VOLUME LVII

AUG 22 1935

NUMBER 2

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

August, 1935

Bence-Jones Protein Excretion and its Effects upon the Kidney. WILEY D. FORBUS, M.D., WM. A. PERLZWEIG, PH.D., I. A. PARFENTJEV, PH.D., AND J. C. BURWELL, JR., M.D. ... 47

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PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore, Maryland

Made in United States of America

BULLETIN

OF

THE JOHNS HOPKINS HOSPITAL

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Correspondence concerning business matters should be sent to The Johns Hopkins Press, Baltimore, Maryland, U. S. A.

This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

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THE JOHNS HOPKINS PRESS

BALTIMORE, MARYLAND, U. S. A.

Entered as second-class matter at the Baltimore, Md., Postoffice. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918

BENCE-JONES PROTEIN EXCRETION AND ITS EFFECTS UPON THE KIDNEY

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Submitted for publication May 25, 1935

In 1920 Thannhauser and Krauss (1) published a careful study of a case of Bence-Jones proteinuria, in a patient who had been under their observation for a considerable period of time and had finally succumbed to his disease—multiple myeloma. At the autopsy there was found an extraordinary and hitherto undescribed kidney lesion, which these two workers regarded as sufficiently similar to those of lipoid nephrosis, mercury poisoning and amyloid disease of the kidney, to justify their grouping this newly found condition of the kidney among the "nephroses." Previous to the publication of the observations of Thannhauser and Krauss numerous clinical observations had been made on the functional state of kidneys which were excreting Bence-Jones protein. Among those who contributed were, Decastello, 1909 (2), Hopkins and Savoy, 1911 (3), Groat, 1916 (4) and Jacobson, 1917, (5). Some of these early observations give the impression that the functional state of the kidney under these conditions is abnormal. In the limited number of cases which were studied both functually and anatomically there is a pronounced inconsistency between the functional and the anatomical disclosures. In reviewing these reports one is impressed by the vagueness of the anatomical descriptions of the kidneys. One may find interesting statistical data covering clinical and pathological nephritis in association with Bence-Jones proteinuria in Decastello's paper (2), all of which, however, must be read with some reservation, in view of the difficulty in interpreting the anatomical description of many of the contributors from whose work the data are taken.

Although attempts to produce experimentally in man and in animals

the "nephritis" of Bence-Jones proteinuria are fairly numerous, one may search in vain for satisfying clinical, pathological, and experimental observations in this field before 1920. The studies made by Thannhauser and Krauss of this date (1) and by Krauss alone in 1921 (6) are sufficiently complete to convince one that functional and anatomical disturbances do take place in the human kidney during and following the time when it is excreting Bence-Jones protein. The interpretation of the anatomical lesions so accurately described by these workers, however, would seem to be in need of some further discussion and perhaps revision, in view of certain observations which we ourselves have made. Hence, the following consideration of the problem is presented.

Our special observations on Bence-Jones proteinuria in man were made in the following case of multiple myeloma which ended fatally and upon which a careful complete clinical and postmortem study was made.

The patient, E. J. (History No. 4721), a white man aged 41, came under observation in the Duke Hospital on May 13, 1931, complaining of pain in the chest and back of about a year's duration. An albuminuria had been discovered at about the time the pain was first experienced, and was present on admission to the hospital. The examination made on admission and throughout the ten days previous to death revealed the following essential conditions: dyspnoea, and tachypnoea; painful respiratory movements of the chest, cyanosis and emaciation; tenderness of the skull on pressure; swollen and tender costochondral junctions; mild secondary anaemia; acid urine of specific gravity of 1.012 containing a faint trace of serum albumin and an abundance of Bence-Jones protein with a few white blood cells. Blood N. P. N. 78 mgm. %, urea N. 58.8 mgm. %, sugar 105 mgm. %, total protein 6.05 gm. %, albumin globulin ratio 1.2, refractive index 1.3490, chlorides (NaCl) 588 mgm. %, phosphorus 7.1 mgm. %, calcium 11.5 mgm. %, urea clearance 14 and 15% of normal, van den Bergh negative. The x-ray studies revealed numerous areas of destruction in the bones of the skull, pelvis, spine and ribs. The temperature ranged between 38° and 39°C., the pulse between 100 and 120 and the blood pressure was 110 over 80. Toward the end there were signs of bronchitis with emphysema and a terminal pneumonia The *clinical diagnosis* was multiple myeloma, chronic bronchitis, emphysema and terminal lobular pneumonia. Death occurred approximately one year after the onset of serious and persistent symptoms and one year after the discovery of Bence-Jones protein in the urine.

Autopsy No. 142—*Anatomical Diagnosis:* Multiple myeloma (plasma cell type) involving skull, left humerus, sternum, ribs, bones of pelvis and the bodies

of the vertebrae; extensive blood formation in spleen; emphysema; chronic bronchitis; hypertrophy of right ventricle; lobular pneumonia; cirrhosis of the liver; obstruction of the kidney tubules by albuminous (Bence-Jones protein ?) casts accompanied by a peculiar tubular and interstitial injury.

Since the pathological findings were characteristic of the usual case of multiple myeloma a description of the various lesions may be omitted except for the alterations in the kidneys.

The gross appearance of these organs was somewhat remarkable. The size of each kidney was not unusual. The changes in the cortex consisted of a considerable degree of irregularity in the striations and some roughening of the subcapsular surface, not unlike that characteristic of the arteriolosclerotic type of nephritis. No alteration of the pelvis was apparent.

In studying the details of the structure of the kidney there were observed features which corresponded fairly well with those in Thannhauser and Krauss' case, the presence of which led these authors to classify their case among the "nephroses" (fig. 1). We were unable to find any abnormality in the glomerular structure even in those areas in which the tubules were most intensely altered.

In connection with the tubular lesion our attention was first attracted by the presence of huge hyaline casts, which, in view of the clinical Bence-Jones proteinuria, were regarded as representing precipitated Bence-Jones protein. We were not able, however, to demonstrate the specificity of the protein. These casts often appeared laminated, there being a peculiar peripheral striated zone with successive layers of material of different density as the center of the hyaline mass was approached, thus producing a close resemblance to corpora amylacea of the prostate. Thannhauser and Krauss referred to similar casts as *Eiweisssteine*. It was not unusual in our case to find surrounding these "albumin stones" a zone of amorphous protein material which seemed to represent a more recent deposit which had not yet become incorporated into the main mass. Surrounding many of the larger casts were numerous mononuclear cells with irregularly shaped nuclei. There were also neutrophilic leucocytes. Desquamated lining cells of the tubules appeared now and then. The most impressive feature of the entire picture was the presence of huge multinucleated giant cells lying in immediate contact with the casts and apparently attempting to encapsulate them. The picture was interpreted as that of a foreign-body reaction. The tubular epithelium was usually absent immediately about the cast; but where the cast was not of sufficient size to cause a great distension of the tubule the epithelium still remained, though often quite flattened by pressure. The tubular obstruction was accompanied by a diffuse infiltration of the adjacent interstitial tissues by lymphoid cells and mononuclear wandering cells like those surrounding the casts within the tubules. It was not unusual to find areas in which many of the tubules were collapsed and surrounded by new connective tissue, but much of the kidney substance remained relatively undisturbed. In the latter areas, especially in the convoluted segments of the tubules, the lining epithelial cells were swollen, quite

granular, and fragile and often contained hyaline droplets. The change in the character of this epithelium was interpreted as one that is perhaps more than a simple cloudy swelling without being an actual necrosis. In other segments of

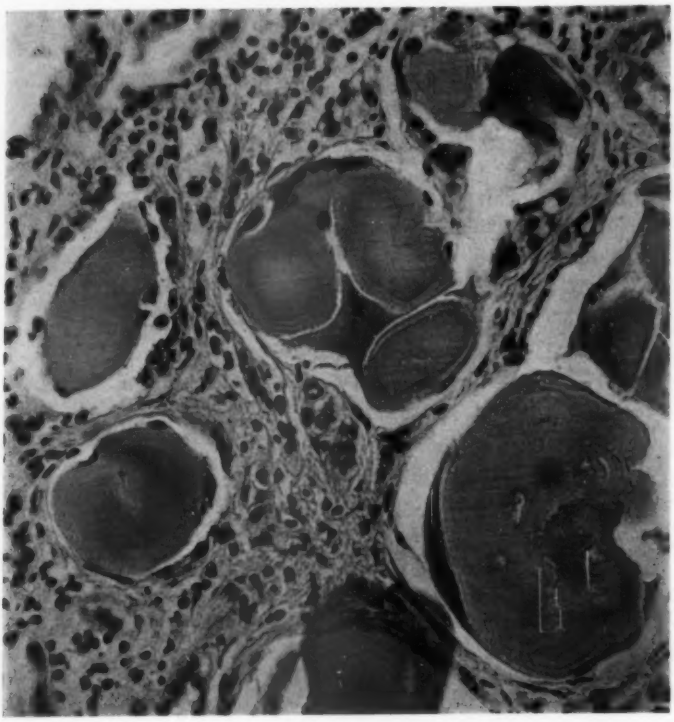

FIG. 1. HIGH POWER MICROPHOTOGRAPH OF A SECTION OF THE CORTEX OF THE KIDNEY FROM CASE NO. 142

The presence of wandering cells and especially the multinucleated giant cells about the protein casts are features of the lesion which are not found in any other form of tubular obstruction and which therefore give to this lesion a certain degree of specificity which is noteworthy.

the tubule the epithelium presented an appearance which might be considered the result of a mild injury, although in degree not in any sense comparable to that of the cells of the convoluted portion.

DISCUSSION

That the kidney lesion just described is one which may be regarded as characteristic of cases in which Bence-Jones protein has been excreted over a considerable period of time seems to us satisfactorily demonstrated not only by the case under discussion but also by our observations in numerous others not described here, and likewise by the observation of Thannhauser and Krauss and more recently of Bell (7). In discussing this lesion it is thought best not to become involved in a maze of terms and definitions such as might arise from the application of the term "nephrosis" to the condition described, as Thannhauser and Krauss did. It seems more useful to analyze what we have found and to attempt to arrive at an understanding of the pathological process involved. Our discussion will therefore deal with three questions.

1. What is the nature and the significance of the changes in the kidney epithelium?

To put it simply, the changes in the kidney epithelium, especially that of the convoluted portion of the tubules, amount to nothing more than a pronounced cloudy swelling. The epithelium is not sufficiently injured to result in desquamation and, in this, one finds a striking contrast with the injury resulting from intoxication such as is produced by mercury, uranium and other specific epithelial poisons. In our case there are so many possible explanations of this minor epithelial damage that it would hardly be justifiable to attribute it to any one cause. It must therefore be regarded as of no definite significance in so far as a possible direct and specific effect of the excretion of Bence-Jones protein is concerned. What we say of our own case we feel may very likely be true of the other cases in the literature.

2. Is Bence-Jones protein specifically toxic for the kidney epithelium?

That Bence-Jones protein does have a decidedly toxic influence upon the body in general has been fairly conclusively shown by Krauss by injection of the protein subcutaneously into himself. The effect is the same as that produced by other foreign proteins. But that there is specific injury to the kidney epithelium has not been demon-

strated. We have no support for the toxic nature of Bence-Jones protein in the case of man other than clinical and pathological observations such as we have found in our own case and the purely clinical observation that a certain number of cases of Bence-Jones proteinuria have an accompanying "nephritis" (Geschickter and Copeland (9)). Such purely clinical observations give us little help, because tests of the kidney functional state rarely give us accurate information as to injury to specific parts of the kidney unit.

It is difficult to see how Bence-Jones protein could pass the glomerulus without producing an injury to the glomerular epithelium, if this same substance will destroy the lining cells of the proximal convoluted tubules, since these cells are closely related in type though perhaps not identical in function. No glomerular epithelial or capillary injury has been observed. It may not be entirely unreasonable, therefore, to say that whatever changes may have taken place in the epithelium of the convoluted tubules were not due to the Bence-Jones protein.

It is not possible to say more about the toxicity of Bence-Jones protein from observations made upon human material. The problem has been attacked experimentally with somewhat conflicting results. While most of the experiments tend to support the view that some injury to the kidney is produced by the administration of Bence-Jones protein to rabbits and dogs, even the best of them, such as those made by Krauss, leave one quite uncertain especially about anatomical injury to the kidney. The epithelial injury, the most commonly described anatomical lesion, is so mild and so much like what we have seen in our human cases, that error in interpretation of the change, especially in the animals used (dogs and rabbits), may easily be made. It is because of this uncertainty that our own experimental study has been undertaken.

3. If there be serious objection to the proposition that Bence-Jones protein is toxic for the kidney epithelium, what explanation is there for the so frequently observed disturbances in function of the kidney in cases of Bence-Jones proteinuria, and just what is the basic nature of the kidney lesion?

The destruction of some kidney tissue and the putting out of function of a great deal more, both apparent in our photographs, affords

an adequate explanation for the functional disturbance which results in a marked nitrogen retention, albuminuria, reduction in urea clearance, and so on. In view of the observed anatomical changes in the kidney it is reasonable to believe that in virtually all cases of prolonged Bence-Jones proteinuria there may develop eventually a disturbance in function of the kidney which is directly due to the excretion of the protein. We are, therefore, inclined toward the view that the statement cautiously made by Geschickter and Copeland, and based very largely on clinical observations, that is, that approximately 61.4% of all cases of Bence-Jones proteinuria are accompanied by a "nephritis" does represent the true state of affairs. We should like, however, to substitute for "nephritis" the phrase "kidney functional disturbance," since the term nephritis implies a great many things which doubtless do not constitute a part of the process for which the Bence-Jones proteinuria is responsible.

The structural changes in the kidney which form the basis of the functional disturbance have, as we see it, a very simple explanation. The lesion is a tubular obstruction which may be favorably compared with that which follows any form of obstruction within the urinary tract whether it be within or without the kidney proper.

This conception of the kidney lesion was suggested by Thannhauser and Krauss when they made use of the term *Eiweisssteine* in describing the Bence-Jones protein casts in the renal tubules. In our preliminary report of this study in 1932 (10) the obstructive conception of the lesion was developed and emphasized as the fundamental process involved. In 1933, Bell (7) also expressed this view of the condition. Although we are dealing here fundamentally with an obstructive phenomenon, there are certain peculiarities about the lesion that differentiate it sharply from other forms of obstructive lesions in the kidney and in fact set this lesion apart as a specific one dependent apparently solely upon a Bence-Jones proteinuria.

We wish to emphasize the cellular reaction about the casts because this is the feature of the lesion which stamps it as a specific one. A picture such as this is not in our experience found in any other form of kidney obstruction. In the usual hydronephrosis there are protein casts, of course, and they vary much in size. But there is no foreign-body reaction about them.

That Bence-Jones protein has characteristics of a foreign substance

capable of producing a foreign protein reaction in cases of Bence-Jones proteinuria was suggested by Perlzweig, Delrue and Geschickter, in 1928 (11), on the basis of the chemical findings in the blood of a case of multiple myeloma associated with hyperproteinemia. This observation is especially interesting since it fits in well with the morphological foreign-body nature of the precipitated protein as indicated by the reaction to the specific protein casts in the kidney tubules.

The Bence-Jones protein tubular obstruction with its accompanying foreign-body reaction is somewhat similar, microscopically, to the lesion produced by bichloride of mercury. It differs from it essentially in that the body which provokes the reaction is entirely different in the two diseases. In the former it is an abnormal protein precipitate, while in the latter it is a mass of coagulated and disintegrating tubular epithelium. By proper comparison the two lesions may be readily differentiated.

Obstruction of the kidney tubules by protein casts must inevitably be followed by damage to the proximal parts of the kidney unit. This means, of course, that the tubular epithelium must suffer injury and finally complete destruction. This is familiar to all and needs no further comment. We refer to the process because it very likely serves as the best explanation of the mild injury to the renal epithelium which has been described by all who have studied the kidneys in cases of Bence-Jones proteinuria, and relieves one of any necessity for assuming that Bence-Jones protein has an injurious direct effect upon the renal epithelium.

In conclusion, it seems that existing observations in man thus far indicate that Bence-Jones protein is not specifically toxic for the renal epithelium, but that its excretion does lead to a disturbance of kidney function through a destructive process in the kidney for which it is directly responsible because of its precipitation in the tubules which permanently obstructs them. The microscopic picture thus produced is a specific one and makes possible the recognition of a previously existing Bence-Jones proteinuria.

EXPERIMENTAL STUDY OF THE FFFECTS OF BENCE-JONES PROTEIN EXCRETION ON THE KIDNEY

In the foregoing discussion of Bence-Jones protein excretion in man and its effect upon the kidney the experimental studies of Krauss,

Decastello and others have been mentioned. None of these experiments have furnished completely satisfactory results. The protein used by some of the experimenters was not purified. In some instances it was contaminated by chloroform and in other instances urine containing Bence-Jones protein was used. The injection material was given directly into the blood in some of the experiments and in others subcutaneously. Rabbits, dogs and man have been used as experimental subjects. The greatest emphasis has been placed upon the chemical changes in the blood. Only in Krauss' experiments were the kidneys studied carefully for anatomical evidence of injury.

After reviewing this subject we felt sufficiently unsatisfied with the recorded data to consider it worth while to repeat the work. This we have done with the hope also of succeeding in producing the characteristic kidney lesion in animals. We hoped thereby to solve especially the problem of the specific toxic character of Bence-Jones protein for the kidney epithelium and to be able to follow step by step the development of the tubular obstructive phenomena. In accordance with this plan the following experiments have been performed.

Experiment I

A. *Dogs.* Three healthy well fed male dogs were isolated in metabolism cages for eight days during which time they were observed and chemical studies of the blood were made. Daily examinations of the urine were also carried out until it was established that the urine in each one was normal. On the eighth day the dogs were given $\frac{1}{4}$ grain of morphine and under ether anesthesia about one-sixth of the left kidney was removed. The right kidney was not disturbed. Because of the relatively small quantity of purified Bence-Jones protein available for the experiments only one of these dogs, No. 1, a white bull mongrel weighing 15 kilos was used for the protein injections. The other two dogs were kept under experimental conditions as controls.

Thirty-eight days after the preliminary operation, injections of purified Bence-Jones protein were begun. During this interim, examinations of the urine and the blood were made and the kidney

function thus shown to be normal. For ten consecutive days the animal received intraperitoneally an injection of purified Bence-Jones protein,[1] the daily dose being approximately 1.296 grams of protein dissolved in salt solution. Chemical examinations of the blood were made at intervals during this period and daily examinations of the urine were made for the presence of Bence-Jones protein, casts, blood, and so on.

Beginning with the eleventh day the protein injections were discontinued for five days and then resumed on the sixteenth day. The injections were continued until the end of the twenty-third day, the daily dose remaining fairly constant. The animal received during the period of 23 days a total of approximately 22 grams of protein.

On the twenty-fourth day the dog was again operated upon and this time the right kidney was removed *in toto*, leaving the previously partially resected left kidney.

Thirty-four days after the removal of the right kidney and the last protein injection the animal was sacrificed and a complete autopsy performed.

[1] Bence-Jones protein used in these experiments was prepared and purified from the urine of the case reported above and from urine from patients in the Mayo Clinic kindly furnished us by Dr. Grace Medes. To facilitate the separation of the B. J. protein from the traces of other proteins present it was found desirable to determine on each batch of urine the optimal pH value (isoelectric point) for its precipitation, using a few drops of the urine in a series of tubes containing 5–10 c.c. of graded buffer solutions. This value in most cases was found to be in the vicinity of pH 5.2. The bulk of the urine was brought to this pH value with acetate buffer, and enough saturated ammonium sulfate solution was added to give 40 per cent saturation with this salt. The precipitated protein was filtered, and washed with ammonium sulfate solution of the same concentration. It was redissolved in the original volume of water and reprecipitated with ammonium sulfate at 60% saturation. The precipitate was redissolved in water, dialyzed in the cold room in viscose bags until the removal of sulfate was complete. The B. J. protein was finally precipitated from the dialyzed solution by the addition of ice-cold alcohol in a total concentration of approximately 60 per cent. The protein was filtered in the cold room and dried in a vaucum desiccator over phosphorus pentoxide. It was a grayish white powder, not easily soluble in water, but more easily soluble in slightly alkaline salt solution. It coagulated at 48–56°C. in the presence of acetic acid, redissolved readily and completely on boiling and reprecipitated on cooling. It behaved similarly with sulphosalicylic, nitric and trichloracetic acids.

Through this procedure, the details of which may be found in Table I, Experiment I, we obtained the following anatomical material for study on the dog:

(1) Normal kidney tissue ($\frac{1}{4}$ of the left) before any protein injections were made.

(2) Supposedly injured kidney tissue (the right kidney *in toto*) through which had been passing Bence-Jones protein for a period of twenty-three days.

(3) Kidney tissue ($\frac{3}{4}$ of the left) which after partial resection had excreted Bence-Jones protein continuously for 23 days, after which it had functioned sufficiently well alone to maintain the animal in good health for a period of thirty-four days.

B. *Rabbits.* Three rabbits were housed in metabolism cages. These animals were treated in the same way as the dogs, except that the entire left kidney was removed for control rather than $\frac{1}{4}$ as in the dogs, and seventy days elapsed between the time of removal of the left kidney and the beginning of the Bence-Jones protein injection and the animal was sacrificed on the twenty-fourth day. Only one rabbit, No. 2A, was given the injections. This animal received intraperitoneally a total amount of 2.493 grams of Bence-Jones protein in doses ranging from 0.112 gm. to 0.3 gm. Frequent examinations of both blood and urine were made.[2]

There was obtained for study from this animal, No. 2A, anatomical material as follows:

(1) Normal kidney tissue (the left *in toto*)

(2) Supposedly injured kidney tissue (the right *in toto*) which had excreted Bence-Jones protein continuously for a period of twenty-three days, during which time the animal was maintained in good health.

The other two rabbits (Nos. 1 and 3) that had received no protein injections were grouped with a third rabbit (No. 4) that had not been operated upon.

[2] The data obtained as shown in detail charts are omitted here and in succeeding experiments for the sake of brevity and in the interest of economy. These data may be obtained on request. The chart here included in connection with the experiment on the dog is representative of those dealing with rabbits and with white mice.

TABLE I

Expt. I

Intraperitoneal injection of purified Bence-Jones protein in salt solution into male Dog 1. Wt. 18 kilos following partial resection of the left kidney for control tissue.

DATE	B.J.P. DOSE	BLOOD N.P.N.	BLOOD URIC ACID	BLOOD CREATINE	WATER INTAKE	URINE VOLUME	URINE SP. GR.	URINE B.J.P.	URINE ALBUMIN	URINE GUAIAC	URINE SEDIMENT	REMARKS
	gms.	*mgm. %*	*mgm. %*	*mgm. %*	*cc.*	*cc.*						
4–8		42	1.4									
4–16	Dog operated on under morphine and ether. One-sixth of kidney (left) resected for control											
5– 8		23	1.6	1.2			1.029	Neg.	Plus		W.B.C. plus Casts plus	Urine data composite of 8 exams. between 5–13 and 5–24
5–24	1.25											
5–25	1.25	30	1.0	1.4		775	1.022	Plus	2 plus	Neg.	Neg.	
5–26	1.25				500	440	1.022	2 plus	Plus	3 plus	Hyal. casts plus	
5–27	1				750	410	1.032	2 plus	Plus	Neg.	Hyal. casts plus	
5–28	1.296	40	0.3	1.4	800	400	1.038	Plus	Plus	Neg.	Hyal. casts 2 plus W.B.C. plus	
5–29	1.296				810	440	1.034	2 plus	Plus	Plus	Hyal. casts 2 plus	
5–30	1.296				790	200+	1.042	Plus	Plus	Plus	Hyal. casts 2 plus	
5–31	1.296	32	0.8	1.4		710	1.024	Plus	Plus	Plus	Hyal. casts plus	
6– 1	1.296					300	1.030	Plus	Plus	Plus	W.B.C. plus Hyal. casts plus	
6– 2	0.864	33	1.8	1.3	760	190	1.030	Plus	Plus	Plus	Hyal. casts plus	
6– 3	0				350	90	1.025	Neg.	Plus	Neg.		
6– 4	0				290	155	1.035	Neg. Plus	Plus	Neg.		
6– 5	0				595	210	1.030	Neg.	Plus	Neg.		
6– 6	0	49	2.2		670	275	1.025	Neg.	Plus	Neg.		

6– 6												Blood NaCl 473
6– 7	0				690	420	1.035	Neg.	Plus	Neg.		Blood Protein Ref. Index 1.3499
6– 8	1.125				360			Neg.	Plus	Neg.	Hyal. casts plus	
6– 9	1.125				855	110	1.035	Plus	Plus	Neg.	Hyal. casts plus	
6–10	0				980	400	1.028	Plus	Plus	Neg.		
6–11	1.125				800	360	1.028	Neg. Plus	Plus	Neg.	Hyal. casts plus	
6–12	1.125					300	1.032	Neg. Plus	Plus	Neg.	Casts plus	
6–13	0				970	170	1.033	3 plus	Plus	Neg.	Casts 2 plus	
6–14	1.125				920	340	1.021	Neg. Plus (?)	Plus	Neg.		
6–15	1.125				680	950	1.015	Neg. Plus (?)	Plus	Neg.		Protein sol. not sterile
6–16	1.125				550	150	1.020	Neg.	Plus	Neg.		
6–17	1.686	46	1.3		500	470	1.032	Plus	Plus	Neg.	Casts plus	Blood NaCl 493. Prot. Ref. Index 1.3481
6–18	Dog operated on. Right kidney removed											
					510	570	1.032	2 plus	Plus	Neg.	Casts plus	
6–19					490							
6–20		36	1.4		500	650	1.032	Neg. Plus.	Plus	Plus	Casts plus	Blood NaCl 512. Prot. Ref. Index 1.3511
6–21					800	265	1.040	Neg.	Plus	Plus	Casts 2 plus	
6–22						250	1.038	Neg.	Plus	Plus	Casts plus	
6–23						75	1.055	Neg.	Plus	2 plus		
7–22	Dog sacrificed and autopsied—weight 15 kilograms											

This group of three rabbits, Nos. 1, 3, and 4, received eleven daily injections of protein intraperitoneally. The daily dose, 0.396 gm., was approximately three times the size of the dose given to rabbit No. 2A and the total quantity of protein was given in half the length of time.

The anatomical material for study obtained from these animals consisted of:

(1) Normal kidney tissue from two rabbits, No. 1 and No. 3, obtailed before protein injections were begun.

(2) Supposedly injured kidney tissue from three rabbits (Nos. 1, 3, and 4) which had been excreting Bence-Jones protein for a period of eleven days and which had received in this time approximately twice as much protein as given to rabbit No. 2A.

Experiment II

This experiment was designed to determine the effect of partial obstruction of the urinary passages upon the precipitation of Bence-Jones protein in the kidney tubules.

A dog (No. 101), weighing about 15 kilos, was isolated and its kidney function studied under normal standard conditions. The animal was then given three daily injections of Bence-Jones protein intravenously. The daily dose was 0.666 gram of purified protein dissolved in salt solution. On the day following the last injection the urine became positive for Bence-Jones protein.

On the fifth day after the last preliminary injection of protein two silver-wire loops were placed around the left ureter at about the midpoint between the kidney and the bladder, the loops being placed about 3 cm. apart.

On the second day after the operation the dog was given 8 c.c. of neoskiadan intravenously for visualization of the kidneys and ureters. By this technique a partial obstruction of the left ureter was demonstrated.

Beginning with the third day after the operation the animal was given, during a period of nine days, a total of 13.13 grams of protein, in daily doses increasing from 1.3 to 2.6 grams. Nine days after the last injection the animal was sacrificed. Daily urine examinations were made and blood chemical examinations at intervals.

From this experiment the following anatomical material was obtained for study:

(1) A right undisturbed kidney through which had been passing Bence-Jones protein for a period of 24 days.

(2) A left partially obstructed hydronephrotic kidney through which Bence-Jones protein had been passing for a period of 24 days.

Experiment III

For this experiment five white mice were employed. On these animals no preliminary study was made. The mice received Bence-Jones protein intravenously, through the tail vein as follows:

Mouse No. 1. This animal was given 10 injections, the dose being 4.5 mgm. of protein dissolved in 0.15 c.c. of salt solution over a period of 25 days. Four days following the last 4.5 mgm. dose a massive dose of 9 mgm. of protein in 0.3 c.c. of salt solution was administered. The animal died thirty minutes after this injection and was autopsied.

Mouse No. 2. This mouse received a total of 12 injections of protein. The first ten were given over a period of 25 days, the dose being 4.5 mgm. in 0.15 c.c. of salt solution. Four days after the last 4.5 mgm. dose an eleventh injection of 6 mgm. was given which was survived. Three days later the twelfth injection, 6 mgm. of protein was given and the mouse died. A complete autopsy was done.

Mouse No. 3. This mouse received twelve intravenous injections, the dose being 4.5 mgm. of protein in 0.15 c.c. of salt solution. These injections were given over a period of 25 days. The animal was accidentally killed. A complete autopsy was done.

Mouse No. 4 and No. 5. These mice received 12 intravenous injections, each time 4.5 mgm. of protein in 0.15 c.c. of salt solution being given, over a period of 25 days. Four days after the last injection of 4.5 mgm. of protein a new series of injections was begun. Beginning with a dose of 4.5 mgm. of protein and gradually increasing to 30 mgm. the protein was administered subcutaneously eight times over a period of 26 days. Samples of the urine of these mice showed the presence of Bence-Jones protein. Although these two mice received 20 injections and a total of approximately 125 mgm. of protein,

they remained alive. They were sacrificed fifteen days following the last and maximum dose, 35 mgm. of protein. Complete autopsies were performed.

From this experiment anatomical material as follows was obtained:

(1) Kidneys from a mouse No. 1, after receiving a total of 54 mgm. of Bence-Jones protein intravenously.

(2) Kidneys from a mouse, No. 2, that had received 57 mgm. of protein intravenously.

(3) Kidneys from a mouse, No. 3, that had received a total of 54 mgm. of protein intravenously.

(4) Kidneys from two mice, No. 4 and No. 5, each of which had survived 54 mgm. of protein given intravenously and an additional 71 mgm. given subcutaneously and both of which were excreting Bence-Jones proteins for fifteen days before they were sacrificed.

DISCUSSION OF EXPERIMENTAL MATERIALS

The tissues obtained at the autopsy on our dog No. 1 were studied both grossly and microscopically. Since our interest is predominantly in the kidney and since the other organs showed nothing of pathological importance, we may confine our discussion to the condition of the kidneys.

The control piece of the left kidney removed at operation showed microscopically what is described by Hutyra (13) as a chronic focal nephritis. This lesion resembles closely the focal cortical scarring familiar to all in the human kidney and which is accompanied by round-cell infiltration but not by any disturbance in function of the kidney. We prefer not to describe this human lesion as a nephritis, nor do we look upon the comparable lesion in dogs as constituting a nephritis. Because of the almost universal appearance of this lesion in dogs and because of the absence of any functional disturbance in the kidney accompanying it, we have disregarded it in our consideration of the possible effects of Bence-Jones protein excretion upon the dog's kidney and indeed also upon the human kidney. It has been our practice throughout our experiments to discontinue the use of animals in which any confusion in interpretation might arise as a result of this spontaneous lesion.

A very careful study of the control block of kidney from Dog 1, especially with reference to the tubular epithelium, has given us a basis for interpretation of the condition of the tubular epithelium in the kidney subsequently removed which allows little chance of error. A careful microscopic comparison of the control block of left kidney with the whole right kidney which had been excreting Bence-Jones protein in considerable quantity for 23 days has failed to show us any significant variation in the anatomical structure of the tubular epithelium and has forced us to the conclusion that, at least in the dog, the excretion *per se* of this foreign protein does not injure the tubular epithelium. This absence of anatomical evidence of injury to the kidney epithelium is emphasized and supported materially by the fact that our dog No. 1 was able to live normally for 23 days after the entire right kidney and one-sixth of the left kidney had been removed, in spite of the fact that the remains of the left kidney had gone through a period of twenty-three days during which relatively large quantities of Bence-Jones protein had been excreted by it. It should be emphasized that the remaining five-sixths of the left kidney which had maintained the animal in perfect health showed no changes in the renal epithelium when the animal was sacrificed. That there was no subanatomical injury to the kidney epithelium is emphasized by a normal blood and urine throughout the period of excretion of the foreign protein.

What we have said in reference to the kidney epithelium in the dog may be said also in the case of the four rabbits No. 2A, No. 1, No.3, and No. 4. We are aware of the fact that these findings are in direct conflict with those of Krauss. The anatomical picture described by him we feel must have some explanation other than that it is the effect of the administration of Bence-Jones protein. That Krauss recognized such a possibility is indicated by his statement that the lesion in his rabbits was due largely to the general intoxication produced by the protein and only in very small part to the passage of Bence-Jones protein through the kidney.

As far as we are able to determine from Krauss' paper the total amount of protein per kilogram of animal administered was practically the same as that given by us. But Krauss' dose interval and total experimental time far exceeds ours. The total quantity of protein

actually in the animal's body at any given time in our experiments therefore far exceeded that in any of Krauss' animals. Because of this we feel that the absence of kidney damage in our animals becomes more convincing as the correct outcome of the experiment.

In our dog No. 1 casts may be found microscopically in a few of the tubules and especially in the medulla in the straight portion of the tubule near its termination in the pelvis. Such casts have a fairly homogeneous structure and are surrounded by wandering cells, a picture quite like that in the human kidney, though the usual accompanying tubular expansion and the local injury to the tubular epithelium from pressure is entirely absent. In the interstitial tissue immediately surrounding the portion of the tubule that contains the cast there is an accumulation of wandering cells such as may be seen also in the human kidney. While this lesion is essentially identical with that in the human kidney, we wish to emphasize the fact that such lesions are extremely rare and apparently purely focal, since we have not found any evidence of injury to the tubule proximal to the situation of the cast. The scarcity of the lesions indicate to us that they are totally without significance in so far as function or general gross character of the structure of the kidney is concerned (See figs. 2 and 3).

Throughout our studies of the problem we have been impressed with the precipitation of the Bence-Jones protein in the kidney tubules as the fundamental feature of the lesions found. Everything else can be explained as secondary phenomena. Albuminous casts are a usual feature of kidneys in which there is a true nephritis and they are an impressive part of the microscopic picture in cases of hydronephrosis. But secondary changs such as occur in connection with the Bence-Jones protein casts are never seen in these more common kidney diseases.

We do not know why the protein precipitates in some tubules and passes on through others, finally making its way into the pelvis. The reaction of the urine does not explain the precipitation. Because of the extraordinary number of casts present in the tubules of chronically, partially obstructed kidneys, the thought occurred to us that obstruction of the tubule, partial or complete, might result in the protein precipitation.

It was this reasoning that led us to perform our second experiment, in which the ureter was partially obstructed by silver-wire loops. Even though we were successful in producing an especially good unilateral hydronephrosis, thus at least favoring a stagnation of urinary outflow from the tubules, we did not produce the microscopic

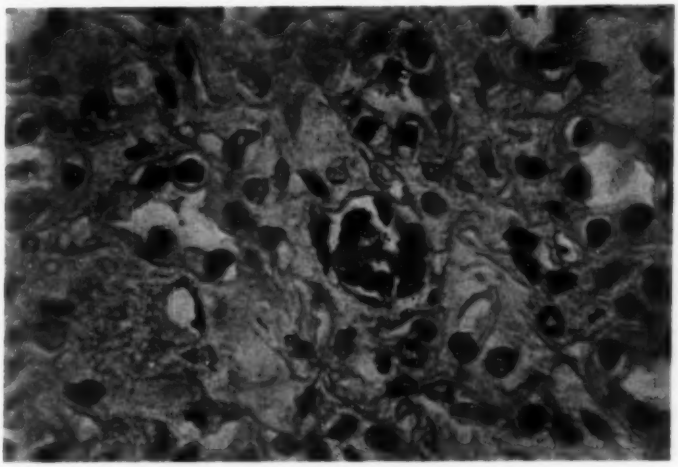

FIG. 2. MICROPHOTOGRAPH OF THE MEDULLARY PORTION OF THE KIDNEY FROM DOG NO. 1

This dog received a total of 22 gms. of Bence-Jones protein over a period of twenty-three days and excreted the protein almost continuously through the kidneys during this time. In the center of the photograph is one of a very few obstructed or partly obstructed tubules which were found. The lesion consists essentially of a protein cast surrounded by wandering cells and is the nearest approach to the lesions produced spontaneously in the human kidney which we have been able to produce experimentally.

picture characteristic of Bence-Jones proteinuria in man. Albuminous casts are present in large number in the hydronephrotic kidney and there is also the usual tubular collapse with interstitial growth of connective tissue. But there is no reaction of wandering cells in the interstitial tissue about the casts and no cell mass surrounding the cast within the tubule. Occasionally, a few mononuclear cells may be seen immediately about the casts.

We do not know that the aforementioned tubular casts are composed of Bence-Jones protein. The animal, our dog No. 101, received large quantities of protein and this appeared in the urine for a period of fourteen days before the animal was sacrificed. The quantity of the specific protein appearing in the urine was, however, quite small and it was accompanied by a much greater quantity of

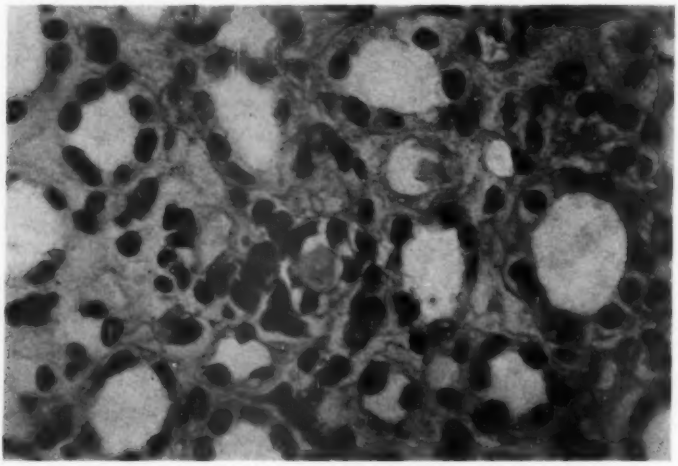

FIG. 3. MICROPHOTOGRAPH OF THE MEDULLARY PORTION OF THE KIDNEY FROM DOG NO. 1

(Compare with fig. 2.) In the center is a tubule obstructed by a protein cast. Surrounding this segment of the tubule is a cluster of wandering cells in readiness to invade the tubule for the purpose of phagocytizing or encapsulating the foreign material. This process may be followed also in the human kidney.

serum albumin. This animal, though reacting quite like all the other animals to the protein injections, seemed to be able to metabolize the foreign protein far better than any other, although the protein was given directly into the blood stream. The dog received as much as 2.6 grams of purified protein at one time. During one period of the experiment, the last four days of injection, a total of 8.9 grams of protein was given directly into the blood. At this time the Bence-Jones protein test of the urine was clearly positive but

relatively large quantities of protein were not excreted. The urine was positive for nine days following the last dose of protein but negative for the three days preceding termination of the experiment.

It appears to us that there was ample time for a characteristic cell reaction to any precipitated foreign protein to have taken place. Assuming that the casts were composed at least partly of Bence-Jones protein, we can give no explanation of the absence of this reaction unless it be that the proportion of Bence-Jones protein to serum protein was so small that the foreign-body character of the cast was not appreciated by the tissues. We have not been able to repeat this experiment because of lack of materials. We feel that this should be done with an animal that cannot metabolize the foreign protein so successfully and therefore would excrete it more profusely, and that the experiment should be prolonged, especially in view of the demonstration in our first experiment, our dog No. 1, that the dog's kidney tissues will react to the presence of the precipitated Bence-Jones protein as does the human kidney tissue.

In view of our failure to produce a lesion in dogs and rabbits quantitatively like that in man we turned to the use of white mice—our experiment No. 3. Here, however, the outcome was the same as with the other animals. While these animals do excrete Bence-Jones protein in the urine following its intravenous injection, our experiment shows that it is done without significant anatomical injury to the kidney.[3]

SUMMARY AND CONCLUSIONS

(1) A case of Bence-Jones proteinuria in man has been studied with special reference to the effect of the excretion of large quantities of the protein upon the kidney.

(2) The excretion of relatively large quantities of Bence-Jones protein by the human kidney is accompanied by precipitation of the protein in the tubules in the form of peculiar casts having a fairly

[3] It may be of interest to recall here that white mice excrete normally a protein in remarkably high concentrations. This protein is not of the serum albumin or globulin type and it appears to belong to the group of chondromucoids. Its origin is completely unknown. Parfentjev, I. A., and Perlzweig, W. A.: *J. Biol. Chem.*, 1933, **100**, 551.

constant structure. These casts, being composed of an abnormal protein substance, completely obstruct the tubule and provoke a foreign-body reaction, the microscopic picture thus produced being apparently specifically indicative of Bence-Jones proteinuria.

(3) The tubular obstruction following the precipitation of Bence-Jones protein results in extensive secondary changes in the kidney unit, leading finally to its destruction and its replacement by scar tissue.

(4) In the human kidney no evidence has been found in support of the idea that Bence-Jones protein is specifically toxic for the tubular epithelium either in a general way or because of the direct physical contact resulting from passage of the protein through the kidney. The injury to the epithelium which occurs is more logically viewed as a change secondary to the tubular obstruction.

(5) Experimentally it has been shown that the kidneys of dogs, rabbits, and white mice are readily permeable to Bence-Jones protein when this substance is introduced intraperitoneally, intravenously or subcutaneously. The amount of protein which is excreted and its appearance time is variable from animal to animal of like or different species.

(6) The injection of Bence-Jones protein intraperitoneally, intravenously, or subcutaneously, is always accompanied by a definite shock to the animal, as indicated by the appearance of vomiting and a general malaise within thirty minutes after the injections. This disappears after approximately an hour at which time the animal becomes normal.

(7) The administration of Bence-Jones protein in large quantities within a few days, and its continuous excretion in considerable quantity in the urine, does not injure the kidney epithelium to such an extent that it can be seen microscopically. Chemical studies of the blood, studies of the urine and clinical observation of the animal during these acute experiments fail to reveal any kidney functional disturbance that is of significance.

(8) In one of our dogs isolated lesions similar to those accompanying human Bence-Jones proteinuria lesion have been produced, but these are of such scarcity that they are regarded as of little or no significance from the standpoint of kidney function. While we have

been able to facilitate the precipitation of Bence-Jones protein in the kidney tubules of another dog by producing a chronic partial urinary obstruction, the subsequent foreign-body reaction to the protein casts, so characteristic of the human kidney did not occur in the animal so treated. It is suggested that this reaction is dependent upon time and that this experiment was of too short duration to permit its development. Its duration was approximately one-half of that of the experiment in which the isolated characteristic lesions appeared.

(9) From our studies of Bence-Jones proteinuria in man it appears that the excretion of this protein may be expected to produce a significant anatomical injury to the kidney, provided the quantity of protein excreted is relatively large and the duration of the proteinuria prolonged. Disturbance in the kidney function will very likely never be serious in any case but some diminution in function will probably occur in all cases. The presence of a moderate persistent elevation of blood non-protein nitrogen and a mild decrease in urea clearance in the presence of a persistent and relatively pronounced Bence-Jones proteinuria would, therefore, most likely establish the clinical diagnosis of a specific kidney lesion and make possible the prediction of the state of the kidneys with a fair degree of accuracy.

LITERATURE

(1) Thannhauser and Krauss: *Deutsches Arch. f. Klin. Med.*, 1920, **133**, 183.
(2) Decastello: *Ztschr. f. Klin. Med.*, 1909, **67**.
(3) Hopkins and Savoy: *J. Phys.*, 1911, **43**, 189.
(4) Groat: *J. Lab. and Clin. Med.*, 1916, **1**, 895.
(5) Jacobson: *J. Urol.*, 1917, **1**, 167.
(6) Krauss: *Deutsches Arch. f. Klin. Med.*, 1921, **137**, 257.
(7) Bell: *Am. J. Path.*, 1933, **9**, 393.
(8) Henke-Lubarsch: Handbuch Der Speziellen Pathologischen Anatomie und Histologie. VI/1, Julius Springer, 1925.
(9) Geschickter and Copeland: *Arch. of Surg.*, 1928, **16**, 807.
(10) Forbus, Perlzweig, and Burwell: *Southern Med. J.*, 1933, **26**, 360.
(11) Perlzweig, Delrue and Geschickter: *J. A. M. A.*, 1928, **90**, 755.
(12) Parfentjev and Perlzweig: *J. Biol. Chem.*, 1933, **100**, 551.
(13) Hutyra: Special Pathology and Therapeutics of the Diseases of Domestic Animals. A. Eger, Chicago, 1926, 3 vols.

ON THE PATHOGENESIS OF LIPOID NEPHROSIS AND PROGRESSIVE GLOMERULONEPHRITIS

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Submitted for publication June 7, 1935

It has been customary for a long time to classify chronic nephritis associated with oedema into two groups of cases, the "primary degenerative" group, nephrosis, and the "primary inflammatory" group, glomerulonephritis. Thus lipoid nephrosis, or genuine nephrosis, has been separated from chronic glomerulonephritis in etiology, in the type of morbid process concerned, and in the pathogenesis of the so-called "lipoid degeneration" of the renal tubules, although the latter is a conspicuous feature of both diseases. In recent years, however, it has been suggested by Marriott (1), Clausen (2), Aldrich (3), Govaerts (4), and Volhard (5) that bacterial infection plays a part in the etiology of lipoid nephrosis as it does in glomerulonephritis, and many who have written on the subject, including Löhlein (6), Dunn (7), Gainsborough (8), Bell (9), Bennett (10), Peters and Van Slyke (11), Bannick (12), Boyd (13), and Christian (14), consider nephrosis to be a form of glomerulonephritis.

Lipoid nephrosis and progressive glomerulonephritis possess in common numerous clinical and anatomical characteristics, and there are few distinctions between them. The exact etiology of neither is known, and no convincing evidence has ever been offered to support the idea that they represent two distinct specific disease processes fundamentally different in character from each other. This opinion, however, is expressed or implied in many of the current textbooks and reference works (15). Holt and McIntosh (16), for example, say that nephrosis, or tubular nephritis, superficially resembles true nephritis. Addis and Oliver (17) describe nephrosis as "degenerative Bright's disease," and consider the renal lesion to be of secondary importance, in contrast to "haemorrhagic Bright's disease," or glomerulonephritis. According to Karsner (18) it is probable that lipoid nephrosis, unlike

glomerulonephritis, is fundamentally a disturbance of protein-fat-lipoid metabolism. The opinion was expressed by Fahr (19), in a recent discussion, that lipoid nephrosis, in contrast to glomerulonephritis, is a metabolic disease which plays no part as a cause of scarring of the kidneys. Aschoff (20), who formerly considered nephrosis to be atypical glomerulonephritis, now looks on the former as a physico-chemical or chemical disturbance of the blood plasma, and includes lipoid nephrosis among the metabolic diseases (21).

Data were recently collected from the study of ten examples of lipoid nephrosis in children which indicated that the differences between nephrosis and glomerulonephritis which have been interpreted in the past to mean the action of two separate disease processes, are in reality differences in degree of identical processes (22). It was shown that the absence of coagulated exudate, scar tissue, and excessive numbers of regenerated cells between capillaries in the majority of the glomeruli constitutes the one fundamental histological distinction between nephrosis and chronic glomerulonephritis, and that this anatomical difference accounts for the absence of continued nitrogen retention in nephrosis. Clinical and histological evidence was described, and experiments were carried out, suggesting that chronic pneumococcal infection may be concerned in the etiology of the cases reported (23).

In view of the differences of opinion concerning the relation between these two forms of Bright's disease, the following case of chronic glomerulonephritis in a child is described in order to illustrate that nephrosis and nephritis are fundamentally alike in pathogenesis. It shows plainly the character of the anatomical lesions that differentiate the kidneys of the two, and makes evident that these anatomical differences account for the essential points of clinical distinction between them.

The case illustrates the statement that nephrosis and nephritis cannot be accurately separated on a histological basis as "degenerative" and "inflammatory" diseases, since in each there are similar "degenerative" changes in the epithelial cells of the kidney, and a similar inflammatory exudate escapes through the glomerular capillaries in both. It also shows that glomerulonephritis is not necessarily "haemorrhagic Bright's disease," for in many cases gross haematuria

may not occur, and even microscopically the scant number of red blood cells which is found may be no greater than in lipoid nephrosis. It is evident from such a case as the following that glomerulonephritis might be regarded as a disease of fat metabolism for exactly the same reasons that have been given in the past to prove that lipoid nephrosis is a metabolic disease. The data from this case suggest also that bacteria other than beta haemolytic streptococci alone may be concerned in the etiology of some examples of chronic glomerulonephritis with oedema.

Most of the clinical and anatomical features of this case, fifteen weeks in duration, are generally described as being characteristic of lipoid nephrosis. For example, there was massive albuminuria, but gross haematuria never occurred. Red blood cells were found microscopically in the urine on only a few occasions. The urine contained casts, leukocytes, and doubly refractive lipoids. Massive oedema was a persistent feature. The blood cholesterol was high, the plasma protein low, and the ratio of albumin to globulin was reversed. There was no hypertension. Evidence of hidden infection was present. The temperature was often elevated. The antra were cloudy (X-ray). Beta haemolytic streptococci, H. influenzae, and pneumococci were cultured from the throat. Pneumococcal septicaemia and peritonitis developed and were fatal. In contrast to lipoid nephrosis, throughout most of the clinical course of this case, the non-protein nitrogen of the blood was slightly but distinctly elevated.

At autopsy the kidneys were large, smooth, and pale. No gross scars or haemorrhages were found. Microscopically, many of the tubules in the cortical rays were wide. The epithelium of some of these tubules was laden with deposits of fat, including doubly refractive lipoids, and fat deposits were also found in glomerular epithelium. The microscopical sections showed a fine network of loosely arranged connective-tissue cells and fibres between numerous capillaries in many of the glomeruli, and the tubules in each cortical labyrinth were consequently atrophied to a corresponding degree. *In summary*, the case was identical with any example of lipoid nephrosis except for a moderate degree of continued nitrogen retention which was probably in the main dependent on intercapillary scars in the glomeruli and atrophy of corresponding tubules. An account of the case in detail is as follows.

CASE OF CHRONIC GLOMERULONEPHRITIS ILLUSTRATING THE FUNDAMENTAL IDENTITY OF NEPHRITIS AND LIPOID NEPHROSIS

Arthur K., white, 6 years old, was admitted to the hospital on March 19, 1934, and died on June 12, 1934. Duration of the disease, about 15 weeks.

History: Some three weeks before admission to the hospital, gradually increasing oedema of the face, arms, and legs was noticed. There was also frequent urination and vomiting.

Course of the Disease: The temperature, pulse and respiration were normal on admission. Extreme generalized oedema, ascites, and hydrothorax on the left side were present. Nothing else abnormal was found on physical examination. Various therapeutic measures directed toward relief of the oedema were used. Diets rich in fruit and vegetables, high protein diet, transfusion with concentrated blood plasma, and intravenous injection of acacia, were all resorted to without any success. Forty-six hours after transfusion the plasma protein had fallen from 3.75 gm. per cent to 3.0 gm. per cent, and the injections of acacia were followed by rapidly increasing oedema. For weeks the temperature was often elevated from 1 to 2°C. Eventually pneumococcal peritonitis developed. The child died 24 hours later.

Temperature: During a stay of 12 weeks and 2 days in the hospital fever was present on at least 2 days of each of 7 weeks. It varied from 0.4° to 2°C.

X-ray Examinations: A month after admission x-ray examinations showed some clouding of the antra.

Bacterial Cultures: From a throat culture made in March, Alpha streptococci and H. influenzae were reported. Early in May (5/7/34) another culture was made. Alpha and Beta haemolytic streptococci and pneumococci, Group IV, were reported. When peritonitis developed, a month later, Pneumococcus type II was grown from the blood and ascitic fluid. At autopsy the blood and peritoneal exudate contained Pneumococcus type II.

Blood Pressure: The pressure varied from 98/64 to 106/70 mm. of mercury (three determinations).

Urine: The volume excreted was generally less than the fluid intake. S. G., 1.018 to 1.050. *Albumin* content (Esbach), 6.5 to 17 gm. per liter. Albumin-globulin ratio (eight determinations): March, 84/16 or 5.2; April, 66/34 or 1.9; 78/22 or 3.5; May, 92/8 or 11.5; 79/21 or 3.7; 73/27 or 2.7; 73/27 or 2.7. Phthalein excretion not tested. Microscopical examination of centrifugalized specimens showed occasional red blood cells on 4 of 10 examinations. Numerous casts and a few white blood cells were usually present, and the urine contained doubly refractive cholesterol esters exhibiting "Maltese crosses" which disappeared on being heated and reappeared on cooling.

Ascitic Fluid: Three examinations. Total protein content varied from 0.17 gm. per cent to 0.24 gm. per cent and the A/G. ratio from 69/31 or 2.2 to 76/24 or 3.2.

Blood: Wassermann negative. Haemoglobin fell gradually from 94 per cent

on admission to 46 per cent six days before death. The red blood cells and leukocytes were counted only on admission, when they were normal. *Cholesterol* (six determinations) varied from 224 to 770 mgm. per cent. *Non-protein nitrogen* was as follows: March 20th, 62 mgm. per cent; March 23rd, 53 mgm. per cent; April 3rd, 79 mgm. per cent; April 11th, 58 mgm. per cent; April 24th, 70 mgm. per cent; May 22nd, 39 mgm. per cent; June 7th, 48 mgm. per cent. *Total plasma proteins:* Varied from 2.3 to 3.9 mgm. per cent. The A/G. ratio was generally inverted.

Autopsy No. 13696: Anatomical Diagnosis: Chronic ethmoid sinusitis and otitis media; subacute ulcerative tonsillitis; chronic glomerulonephritis; generalized oedema; ascites; hydrothorax (left); septicaemia and peritonitis (Pneumococcus type II).

Summary of Gross Lesions. External appearance: There is very marked generalized oedema. *Abdominal and Pleural Cavities:* The abdominal cavity contains 1150 cc. of cloudy fluid, without any fibrinous exudate. Smears show myriads of bacteria resembling pneumococci. There are 450 cc. of clear fluid in the left, and 90 cc. in the right pleural cavity. *Heart:* Normal in size, weighing 105 grams. Near the apex of the left ventricle there is an opaque yellow area with vague outline, 1 cm. in diameter. It extends nearly through the wall of the ventricle. The coronary arteries look normal. *Lungs:* The lower lobe of the left lung is atelectatic. There is no pneumonia. *Liver:* The liver is large. It weighs 900 grams. The lobules are conspicuous. The periportal areas are grayish-yellow. *Spleen:* The gross appearance is that of the acute splenic tumor. *Intestines:* The mucosa and submucosa are oedematous. The oedematous layer in parts of the colon is 6 mm. thick. *Kidneys:* Each kidney weighs 100 grams. They are enlarged. The surfaces are smooth, yellow in color. No haemorrhages are seen. The cortical striae are straight. There is a pronounced contrast between the yellow color of the cortex and the pale red medullae. Fresh smears from the scraped surface show one or two doubly refractive lipoid bodies, typical cholesterine esters with double crosses, in each high-power field of the polarizing microscope. *Aorta:* The intima is smooth and pale. There are no visible fat deposits. *Tonsils:* The tonsils are moderately enlarged. One is obtained intact. It shows small gray opaque rings on the surface around the opening of the crypts. *Cranial sinuses:* No distinct frontal sinuses are found. The mucosa of one antrum is taken for section. The antra, sphenoid and ethmoid sinuses show no gross exudate. *Ears:* The temporal bones, containing the middle and inner ears, are removed for section.

Summary of Microscopical Lesions. Ears: In each middle ear and in the mastoid cells on each side there are rather large clumps of leukocytes consisting of lymphoid cells and mononuclear phagocytes. No bacteria can be stained. *Ethmoid Sinus:* The mucosa of the lining is intact. The submucosa is infiltrated with numerous plasma cells and occasional polymorphonuclear leukocytes. No bacteria are found. *Tonsils:* Some of the crypts contain masses of necrotic desquamated epithelium, permeated with Gram-positive cocci. Other crypts contain fibrinopurulent exudate in which there are very numerous Gram-positive cocci. On the external surface and in crypts are small areas of necrosis and ulceration of

the epithelium, covered with fibrinopurulent exudate. In other places the squamous cell lining is densely infiltrated with polymorphonuclear leukocytes and lymphocytes. Some of the bacteria stained are cocci in short chains. Others are larger diplococci. There are also clumps of large cocci resembling staphylococci. *Abdominal and Retroperitoneal Tissues:* The peritoneum over the intestine, and in the pelvis, shows infiltrations with polymorphonuclear leukocytes and other cells. In the retroperitoneal tissues about the adrenal and pancreas the same variety of cells is found, together with many pneumococci and fibrinous exudate. *Thoracic Muscles:* Numerous foci are found in the muscle, consisting of necrotic fibres, pneumococci, and polymorphonuclear leukocytes. *Colon:* The submucosa is very oedematous. *Aorta:* Normal. *Liver:* Many liver cells throughout each lobule contain fat in the form of very fine globules, most abundant in the periportal areas. *Spleen:* There are hyaline fat-laden segments in rather numerous arterioles.

Heart: Near the left apex there is an old hyaline scar with irregular borders. In the epicardium nearby a branch of the coronary artery shows an eccentric cellular mass of scar tissue. The muscular wall is intact and there are no vacuoles or crystals of fat.

Kidneys: The cortex shows no distortion of the architecture. That is, the tubules are intact, without interruption. Each cortical labyrinth, with glomeruli and convoluted tubules, is well preserved, and the architecture of the cortical rays is normal.

The majority of the *glomeruli* contain connective-tissue cells and fibres loosely arranged between the capillaries. The fibres stain pink with van Gieson's stain, blue with Mallory's connective-tissue stain. The nuclei of glomerular epithelium are conspicuous and stain deeply with haematoxylin. Occasional tufts of capillaries are found which are covered over by a continuous layer of epithelial cells, and some of these are adherent to the parietal epithelium. There are no large crescents but in a few glomeruli thin clots of exudate containing fibrin can be found between capillaries or in the outer crevice of Bowman's capsule. Very small numbers of mononuclear leukocytes and polymorphonuclear cells can be found in a few glomeruli. Particles of neutral fat and doubly refractive lipoids are present in small quantities in some epithelial cells of a few glomeruli. Some of the endothelial nuclei are larger than normal, but the capillaries are not obstructed by either cells or thrombi.

The tubules in each *cortical labyrinth* are in general smaller than normal. They are lined with thin epithelial cells whose cytoplasm stains deeply, rather purplish in color. Many nuclei are very large, and some cells have several nuclei. Other nuclei are pyknotic, and occasional necrotic cells can be found. There are very few casts in these tubules. Between the tubules there is a little scar tissue, rather loose and delicate, associated in places with small collections of mononuclear leukocytes.

The tubules in the *cortical rays* are wide. Some are lined with large, pale, vacuolated epithelial cells. In others the lining cells are very thin, with coarse granular cytoplasm. Few casts are found in tubules of the cortical rays, and in a

few tubules there are occasional small collections of leukocytes. In two sections of the kidneys, only one tubule filled with red blood cells is found.

Fat stains (Scharlach R) show a moderate amount of neutral fat in most of the epithelial cells lining the small tubules in each cortical labyrinth, and there are patches of large dilated tubules in the cortical rays with abundant fat deposits in the epithelium. The tubular epithelium also contains a moderate amount of doubly refractive lipoid (cholesterol esters exhibiting Maltese crosses).

SUPPOSED SPECIFICITY OF (A) TUBULAR "DEGENERATION" IN LIPOID NEPHROSIS AND (B) "INFLAMMATION" OF THE GLOMERULI IN NEPHRITIS

A single histological change in the renal tubules in lipoid nephrosis, the so-called "lipoid degeneration," has been described so often and so graphically as to leave the impression that it is a particularly characteristic or even specific lesion. This impression has been fortified by failure to appreciate the fact that identical lesions are also commonly found in the tubular epithelium in chronic glomerulonephritis, although the presence of fat deposits and doubly refractive lipoids in the kidneys of glomerulonephritis was described as early as 1902 by Kaiserling and Orgler (24), and later by Löhlein (25, 26, 27) and others. It has always been taught, without any real evidence for the idea, however, that this change is "primarily degenerative" in lipoid nephrosis, "secondary" in nephritis.

For a long time it has been well known that leukocytes of one kind or another may be found in the urine, glomeruli, renal tubules, and in the interstitial tissue of the kidney in varying numbers in any case of Bright's disease, regardless of etiology and terminology, and it seems to be self-evident that the blood cells, both red and white, and the plasma proteins, albumin and globulin, which appear in the urine in varying concentration from time to time in any case, may be regarded as an inflammatory exudate in lipoid nephrosis with as much reason as in glomerulonephritis.

Nevertheless, following the early teaching of Friedrich Müller (28), Volhard and Fahr (29), and Munk (30), nephritis was divided into two groups of cases, the one, glomerulonephritis, regarded as primarily an inflammation of the glomeruli, the other, nephrosis, said to be primarily a "degeneration" of the tubules. Identical histological lesions (fat and lipoid deposits in tubular epithelium) were made "secondary"

in one group, and "primary" in the other, although the exact etiology of the cases in both groups was very uncertain. As a result of these rather artificial distinctions a confusion of terms and theories grew up, and the essential differences between nephrosis and nephritis were submerged in a maze of words and forced "concepts."[1] This classification was adopted, however, in spite of the well-known facts mentioned above, namely, that leukocytes and fat deposits in varying quantity are found in the kidneys in each group of cases, and that blood cells and plasma proteins constantly exude through the glomerular capillaries to appear in the urine in both glomerulonephritis and lipoid nephrosis. It became a doctrine that red blood cells never appear in the urine in lipoid nephrosis, although microscopical haematuria was described in some of the first cases ever reported (31), as well as in many later reports. The presence of leukocytes in the kidney and urine in nephrosis, and the fact that equally scant numbers are found in many cases of glomerulonephritis, were not fully appreciated. It was frequently repeated, without any good evidence to support the statement, that the "degenerative" changes (fat and lipoid deposits) in the kidney of nephrosis were "primary," in contrast to their "secondary" mode of origin in nephritis. Thus the theory has been perpetuated, even down to the present time, that lipoid nephrosis is a "degenerative" disease, while glomerulonephritis, in contrast, is said to be something quite unrelated, primarily an "inflammatory" disease (32). The fact is, however, that the so-called "lipoid degenerations" in tubular and glomerular epithelium, and *similar* inflammatory exudates from the glomeruli, occur in both diseases. The pathogenesis of the lipoid deposits is not clear, and it has never been shown that they are caused by different mechanisms in nephrosis and nephritis.

The example of nephritis described above, and similar cases reported in the literature (9, 10), together with the studies of lipoid nephrosis

[1] Some of the multitude of terms applied to nephrosis and nephritis with oedema are as follows: Nephrodystrophy, tubular degenerative nephropathy, lipoid dystrophy of the kidney, cryptogenic nephrosis, pure lipoid nephrosis, genuine nephrosis, uncomplicated nephrosis, tubular nephritis, glomerulonephrosis, parenchymatous nephritis, burnt-out glomerulonephritis, nephrotic syndrome, nephrotic nephritis, nephritis with nephrotic *Einschlag*, mixed types of nephritis, nephritis superimposed on nephrosis, *et cetera*.

referred to (22), leave little doubt that the fundamental histological differences between nephrosis and chronic glomerulonephritis depend on the coagulation of exudate, and growth of new cells and connective-tissue fibers in the glomeruli between the capillaries. Changes in the permeability of glomerular capillaries, and the escape of exudate containing plasma proteins and blood cells in varying concentration constitute inflammation whether occurring in nephrosis or nephritis. The inflammatory nature of this process is evidently independent of the fate of the exudate, *i.e.*, coagulation and organization between the glomerular capillaries in some cases. Fat and lipoid deposits, the so-called "degenerative" changes, as well as evidence of inflammation of the glomeruli, whether the latter be found microscopically represented by cells in the tissues, or measured in the urine as exudate of cells and proteins, are common to both nephrosis and progressive glomerulonephritis. All of these phenomena differ in the two diseases in degree, and perhaps in etiology. But the urine of nephrosis and of many cases of glomerulonephritis, on comparison, may exhibit quantitative differences in the cellular and protein exudate which are slight, and equal variations occur from time to time in most cases of chronic nephritis.

THE RELATION BETWEEN GLOBULIN IN THE URINE AND COAGULATION OF EXUDATE IN THE GLOMERULI

The protein in the urine, whether of lipoid nephrosis or chronic glomerulonephritis, consists of albumin and globulin of the blood plasma, as everyone knows, although the term albuminuria is loosely used to indicate proteinuria, and the usual tests (heating, and Esbach precipitation) are tests for protein, not for albumin alone. The urine from cases of nephrosis examined by Hiller, McIntosh, and Van Slyke (33) contained small proportions of globulin, and the albumin-globulin ratio was therefore high, always greater than 5, and usually greater than 10. In cases of glomerulonephritis which they studied the proportion of globulin in the urine was higher and the albumin-globulin ratio therefore lower than in nephrosis, lying between 3.5 and 8.9. Similar results have been reported by Geill (34). The urine from the example of nephritis described above generally contained a considerable proportion of globulin. The albumin-globulin ratio was deter-

mined on eight occasions, usually at rather long intervals. On six occasions the globulin concentration was high and the ratio low (66/34 or 1.9; 70/30 or 2.3; 78/22 or 3.5; 79/21 or 3.7; 72/27 or 2.7; 73/27 or 2.7). The albumin-globulin ratio was greater than 5 on two determinations (92/8 or 11.5 and 84/16 or 5.2), when the globulin content of the urine was evidently low. This evidence suggests that there is some relation between high globulin content of the urine, and therefore probably high fibrinogen content, and the formation of the clots and scars in the glomeruli which are associated with progressive nitrogen retention in the blood. The low concentration of globulin in the urine of nephrosis, and the absence of clots and scars in the glomeruli (22), seem equally significant.

The excretion of fibrinogen, one of the globulins, is only one of the factors concerned in the coagulation of exudate, however, and all of the other necessary substances, such as calcium, prothrombin, and thrombokinase, must doubtless be present in the glomerular spaces in proper proportion, otherwise the fibrinogen, when excreted, will appear in the urine along with other globulins and albumin, and clots may not form in the glomeruli. The quantity of thrombokinase, formed perhaps as the result of necrosis of tissue, is probably very important, and may well vary with different etiological agents. Whether or not the excretion of globulins through the glomerular capillaries may lead to scarring and hyalinization of the glomeruli by any mechanism apart from that dependent on the clotting of fibrin, is as yet undetermined, but it is suggested by the high proportion of globulins in the urine and the hyaline deposits in the glomeruli in amyloid disease.

ON THE ORIGIN OF GLOMERULAR SCARS

The individual glomerular capillaries in the above case of nephritis, as also in lipoid nephrosis (22), do not show definite microscopical changes to account for the escape of cells and plasma through them. They contain no thrombi, nor are they occluded by endothelial cells or leukocytes, although some leukocytes are found both within and outside the capillaries, and though some endothelial as well as epithelial nuclei are larger than normal. The majority of the newly formed cells, and the connective-tissue fibres, lie between capillaries,

outside the capillary lumen. The new cells and fibres long associated with the term "intracapillary nephritis," recently emphasized by McGregor (35) and Bell (9) as characteristic of all glomerulonephritis, have been shown by MacCallum (36) to lie outside the lumen of the glomerular capillary, between adjacent tufts. In general, the exact origin of all of the scar tissue in the glomeruli in any case is open to some question, and it is uncertain, in these cases of chronic diffuse nephritis, to what extent connective-tissue cells and fibres may be stimulated to grow between the glomerular capillaries in the absence of a network of fibrin filaments, along which fibroblasts grow so readily in the formation of scar tissue in any part of the body (37). Since it appears, however, that glomerular scars develop readily in those cases in which globulins are excreted in the urine in high concentration (glomerulonephritis), and since strands of fibrin can be identified between some of the glomerular capillaries in the present case, as in many other cases of progressive nephritis, it is clear that mature scars are at least in part formed by the organization of glomerular exudate.

PROGRESSIVE SCARRING OF THE KIDNEY AND URAEMIA

It has often been questioned whether or not lipoid nephrosis may lead to the development of diffuse scarring of the kidneys and uraemia. Bell states that uraemia does not develop and that the patient with lipoid nephrosis either recovers or dies from intercurrent infection (9). Leiter, too, says that uraemia never occurs in lipoid nephrosis (38), but maintains that nephrosis is often complicated by a different, unrelated disease, nephritis. Bennett *et al.* (10), on the other hand, suggest that cases of lipoid nephrosis may exist for a time as such, later to develop signs and symptoms of progressive nitrogen retention and hypertension, and they describe such cases. As a matter of fact, these examples are not uncommon, and probably comprise many of those known as nephrotic nephritis, mixed types, *et cetera*.

The absence of uraemia or its development probably does not depend, however, simply on the presence of some rigidly defined disease, "degenerative" nephrosis, or "inflammatory" glomerulonephritis. Besides the influence of functional factors which may prevent excretion of nitrogen, the pathogenesis of uraemia is related, rather, to the

action of all of the factors which cause anatomical changes, especially to the existence of clots and scars formed in the presence of glomerular exudate containing cells, albumin, and globulin. In order, therefore, to determine whether in any case glomerular scars and uraemia will develop, it is important to know (1) what agents cause glomerular damage, and quantitative variations in the exudation of blood cells and proteins; (2) what factors determine whether or not part of the escaping exudate will clot, be organized, and leave scars; and (3) what are the differences in etiology, if there are differences, between the cases in which coagula, scars, and uraemia tend to develop readily (chronic glomerulonephritis), and those which may exist for long periods without these phenomena (lipoid nephrosis). Either different etiological agents, or the same agent acting with different degrees of intensity, might account for variations in the fibrinogen content of the exudate, concentration of thrombokinase formed, or, in other words, for the presence or absence, duration and concentration of the factors which cause clots and scars to form between glomerular capillaries through which blood cells and plasma proteins escape.

PATHOGENESIS

After "genuine lipoid nephrosis" was theoretically established as a "purely degenerative" disease, essentially different from "inflammatory" glomerulonephritis, new hypotheses appeared from time to time to explain the pathogenesis of nephrosis, while evidence steadily accumulated which suggested that glomerulonephritis was related in etiology to bacterial infection. The theories advanced have been reviewed and discussed by Leiter (15). Many of them are open to serious objection for at least three reasons. First, the very fact that they were proposed rests on the theory, for which there was never any real evidence, that nephrosis was anatomically a "degenerative" process, nephritis an unrelated "inflammatory" process. Second, while most of the clinical and anatomical characteristics of nephrosis and nephritis are common to the two, the theory that the diseases are specific and different in kind fails to consider the fact that in each disease abnormal anatomical lesions and physiological processes occur, most of which are identical in kind but different in degree. In the

third place, the theories offered to explain the metabolic nature of lipoid nephrosis could be applied with equal reason to explain the pathogenesis of chronic glomerulonephritis.

For example, the theory of Epstein, that lipoid nephrosis is a "metabolic" disease, "diabetes albuminuricus," postulates the development of abnormal plasma proteins which are then excreted by the kidney. Glomerulonephritis might be considered "diabetes albuminuricus" with equal justification. The theory cannot explain the oliguria of nephrosis, and is, of course, incompatible with the excretion of leukocytes and red blood cells which occurs in nephrosis. It has been stated repeatedly that the urine in nephrosis contains no blood, although microscopical haematuria was described by Munk from the beginning (31); and it is well known that gross haematuria may not occur in some cases of glomerulonephritis. The appearance of gross haematuria in any case is generally temporary, and red blood cells are often discovered only under the microscope. It has been shown, too, by Hewitt (39, 40), and by Cavett (41) that the proteins in the urine of lipoid nephrosis and nephritis, when purified, are physically and chemically indistinguishable from normal serum proteins. Data have also been collected showing that abnormal microscopical haematuria does occur in many cases of lipoid nephrosis (22), exactly as in many examples of chronic glomerulonephritis.

Actually, no valid clinical, chemical, or bacteriological evidence has been found which shows that lipoid nephrosis and chronic glomerulonephritis are diseases of fundamentally different nature. The anatomical distinctions between them are related especially to quantitative differences in the exudate, and to its fate (coagulation and organization) in passing through the glomeruli. All of the known *facts* indicate that both diseases are closely related in pathogenesis to foci of bacterial infection outside the kidney which causes damage to glomerular capillaries and to the epithelial cells of glomeruli and tubules. As a result of *glomerular* damage, two distinct processes may occur. First, exudate consisting of leukocytes, red blood cells, and plasma protein escapes from the capillaries, and can be traced under the microscope directly from the glomeruli into the tubules. In both nephrosis and nephritis most of the exudate appears in the urine. The number of cells in the exudate, and the concentration and proportion

of albumin and globulin are variable in every case. Second, at times when the exudate is rich in globulin, clots and scars may form between capillaries of the glomeruli, and capillary thrombi may also develop. This second process may not occur at all, or not for a long time. Long continued or progressive nitrogen retention is therefore absent and the condition is called nephrosis. Clots and scars may, however, develop in many glomeruli throughout the kidneys, and this may take place slowly or rapidly. Under these conditions waste nitrogen is eventually retained in the blood, and the disease is called nephritis. The development of this process and the extent to which it may occur depend on many factors. Of these the amount of fibrinogen, and perhaps the quantity of other globulins in the exudate, are important. With increasing numbers and density of scars, elevation of blood pressure generally appears. The exact pathogenesis of hypertension under the circumstances, as everyone knows, is far from clear. It is evident, however, that in chronic Bright's disease, including lipoid nephrosis, one mechanism, glomerular damage, is responsible for the presence of blood cells and proteins in the urine. The formation of clots and scars in the glomeruli is a separate mechanism, dependent upon the first, it is true, but quite distinct from it, and evidently one of the chief factors responsible for continued and progressive nitrogen retention. Until nephritis can be classified on the basis of etiology, therefore, it seems unjustifiable to regard nephrosis and glomerulonephritis as separate disease processes.

Very little is known concerning the rôle of the altered *tubules* in the pathogenesis of the manifestations of nephrosis and glomerulonephritis, whether the changes in the cells are shown as cloudy swelling, fat deposits, atrophy or necrosis. What the functional disorders are that may be caused by damage to epithelial cells remains to be investigated. It seems possible, but is not certain, that the oliguria of nephrosis and polyuria of advanced chronic nephritis may be related to altered tubular epithelium. The one evident anatomical fact concerning the tubules is that "degeneration" of epithelial cells (cloudy swelling, lipoid and fat deposits, and necrosis) occurs in chronic glomerulonephritis as frequently and often with an equal degree of intensity as in lipoid nephrosis.

ETIOLOGY

It was suggested recently, from the study of a series of cases of lipoid nephrosis in children, that nephrosis and glomerulonephritis may be similar in etiology as well as in clinical and anatomical manifestations (22). There was evidence in these cases that focal pneumococcal infection was concerned in the etiology of the disease, and this was given support by the experimental production of nephrosis in animals injected with small doses of pneumococcal toxin (23). In the example of nephritis described here, there was clinical and anatomical evidence of chronic infection of the tonsils and cranial sinuses in which pneumococci as well as other bacteria were probably concerned. Alpha and beta streptococci, influenza bacilli, and pneumococci were grown from the throat. In microscopical sections small ulcers in the tonsils and exudate in the crypts were found, together with Gram-positive diplococci, cocci in chains, and in clumps. The mucous membrane of the ethmoid sinuses showed definite chronic inflammation, although no bacteria could be demonstrated. In this case, with definite diffuse glomerular scars and moderate nitrogen retention, and in the examples of lipoid nephrosis without appreciable glomerular scars and retention of nitrogen, as described before (22), the evidence of chronic bacterial infection, the sites of infection, and the variety of bacteria found in the infected tissues are apparently quite similar. It is difficult to determine the rôle of the streptococci, of pneumococci, or of other bacteria in the pathogenesis of these cases. Since pneumococci can be demonstrated in the foci of infection and since various forms of nephritis may be reproduced in animals by means of pneumococcal toxin (23), it seems probable that chronic pneumococcal infection is concerned in the etiology of certain examples of chronic glomerulonephritis.

SUMMARY

A case of chronic glomerulonephritis is described which resembled lipoid nephrosis in practically every particular. There was massive oedema. The urine contained large amounts of protein, many casts, doubly refractive lipoid crystals, leukocytes, and very few red blood cells. The plasma proteins were low and the albumin-globulin ratio

was inverted. The blood cholesterol was very high. At autopsy the kidneys were large, smooth, and yellow, without gross scars. There was microscopical evidence of damaged tubular and glomerular epithelium, and lipoid deposits were plentiful.

The case differed from lipoid nephrosis in the presence of continued nitrogen retention of moderate degree, and this was generally accompanied by the excretion of rather large amounts of globulin in the urine. Microscopically, a fine network of scar tissue was found between many glomerular capillaries. The corresponding tubules were atrophied, and there were fresh threads of fibrin between a few capillary loops. These data suggest a significant relationship between the globulin content of urine, the progressive formation and organization of clots between capillaries of the glomeruli, and the consequent development of extensive glomerular scars and uraemia in chronic nephritis.

The case shows that essentially the same processes are concerned in the pathogenesis of lipoid nephrosis and chronic glomerulonephritis. The classification of nephritis on the basis of tubular "lipoid degeneration" and glomerular "inflammation," in so far as it implies the accurate description of specific and fundamental differences between lipoid nephrosis and glomerulonephritis, is fallacious. Identical lipoid deposits are found in the kidney and urine in both. In each condition inflammatory exudate of variable quantity and composition escapes from the glomeruli, and most of it passes out in the urine. The chief anatomical differences between nephrosis and chronic glomerulonephritis are closely related to the fate of the inflammatory exudate, *i.e.*, the formation of clots, and the growth of scar tissue, between the capillaries of the glomeruli. The concentration of globulin excreted in the urine, at least in so far as it may favor the excretion of fibrinogen, is a significant factor in promoting coagulation of exudate and the formation of scars in the glomeruli. It seems evident that in addition to the quantitative differences in cellular and protein content of the glomerular exudate and urine in nephrosis and nephritis, there are a number of other factors concerned in determining the presence or absence of glomerular scars. The fact is also obvious that the widely accepted distinction between nephrosis, a "primary degenerative disease," and glomerulonephritis, an inflammatory disease of the

glomeruli, neither expresses these quantitative differences which are of fundamental significance in the pathogenesis of anatomical lesions in the glomeruli and tubules, nor does it describe accurately the real histological differences which do exist in the kidneys in these two forms of bilateral diffuse nephritis.

There is evidence to suggest that chronic pneumococcal infection may be concerned in the pathogenesis of certain cases of chronic glomerulonephritis.

BIBLIOGRAPHY

(1) MARRIOTT: *Med. Clinics of North Amer.*, 1924, **7**, 1413.
(2) CLAUSEN: *Am. J. Dis. of Child.*, 1925, **29**, 581, 587, 594.
(3) ALDRICH: *ibid.*, 1926, **32**, 163.
(4) GOVAERTS AND CORDIER: *Bull. Acad. Roy. Méd. Belgique*, 1928, **8**, 510.
(5) VOLHARD: Handbuch der inneren Medizin, 1931, 6, II, 1056, Julius Springer, Berlin.
(6) LÖHLEIN: *Deut. med. Wchnschr.*, 1918, **44**, 1187.
(7) DUNN: *Glasgow Med. Jour.*, 1924, **101**, 64.
(8) GAINSBOROUGH: *Quart. J. Med.*, 1929, **23**, 101.
(9) BELL: *Am. Jour. of Path.*, 1929, **5**, 587.
(10) BENNETT, DODDS, ROBERTSON AND BAKER: *Quart. Jour. of Med.*, 1930–31, **24**, 239.
(11) PETERS AND VAN SLYKE: Quantitative Clinical Chemistry, Interpretations, 1932, 670, Williams & Wilkins, Baltimore.
(12) BANNICK: *J. A. M. A.*, 1934, **102**, 172.
(13) BOYD: Text-book of Pathology, 1934, 630. W. B. Saunders & Co., Philadelphia, Pa.
(14) CHRISTIAN: Cecil's Text-book of Medicine, 1934, 979, W. B. Saunders & Co., Philadelphia.
(15) LEITER: *Medicine*, 1931, **10**, 235.
(16) HOLT AND MCINTOSH: Holt's Diseases of Infancy and Childhood. 1933, 683, D. Appleton & Co., New York.
(17) ADDIS AND OLIVER: The Renal Lesions in Bright's Disease, 1931, 91, T. B. Hoeber, N. Y.
(18) KARSNER: Human Pathology, 1931, 741, Lippincott & Co., Philadelphia.
(19) FAHR: Henke-Lubarsch, Handbuch d. Path. Anat., 1934, 6, II, 815, 829, 835, Springer, Berlin.
(20) ASCHOFF: Cited by Fahr in Handbuch d. Path. Anat., 1934, 6, II, 830, Springer, Berlin.
(21) ASCHOFF: Pathologische Anatomie, 1928, 2, 484, G. Fischer, Jena.
(22) BLACKMAN: *Bull. Johns Hopkins Hospital*, 1934, **55**, 1.
(23) BLACKMAN: *ibid.*, 1934, **55**, 85.

(24) KAISERLING AND ORGLER: *Virchow's Arch.*, 1902, **167**, 296.
(25) LÖHLEIN: *Verhandl. d. deut. path. Gesellschaft*, 1904, **8**, 33.
(26) *Idem: Virchow's Arch.*, 1905, **180**, 1.
(27) *Idem: Ergebn. inn. Medizin u. Kinderheilkunde*, 1910, **5**, 411.
(28) MÜLLER: *Verhandl. d. deut. path. Gesellschaft*, 1905, **9**, 64.
(29) VOLHARD AND FAHR: Die Brightsche Nierenkrankheit, Berlin, 1914.
(30) MUNK: *Med. Klinik*, 1916, **12**, 1019, 1047, 1073.
(31) *Idem: Ztschr. f. klin. Medizin*, 1913, **78**, 1.
(32) LEITER: *Medicine*, 1931, **10**, 141, 235.
(33) HILLER, MCINTOSH AND VAN SLYKE: *Jour. Clin. Investigation*, 1927, **4**, 235.
(34) GEILL: *Ztschr. f. klin. Medizin*, 1929, **110**, 334.
(35) MCGREGOR: *Am. Jour. Path.*, 1929, **5**, 559.
(36) MACCALLUM: *Bull. Johns Hopkins Hosp.*, 1934, **55**, 416.
(37) *Idem:* Text-book of Pathology, 5th Ed., p. 176. W. B. Saunders & Co., Phila.
(38) LEITER: *Medicine*, 1931, **10**, 158, 141, 235.
(39) HEWITT: *Biochem. Jour.*, 1927, **21**, 1109.
(40) *Idem:* Lancet, 1929, **1**, 66.
(41) CAVETT: *J. Biol. Chem.*, 1930, **16**, 87.

EXPLANATION OF PLATES

(Photographs by Mr. Milton Kougl)

FIG. 1. Autopsy No. 13696. The kidneys are smooth and very pale.

FIG. 2. No. 13696. Haematoxylin and eosin stain. × 90. There is a delicate network of connective-tissue cells and fibres between capillaries of the glomeruli. The corresponding tubules in the cortical labyrinth are atrophied, and there is some scar tissue between them. The tubules in the cortical rays (in the upper left and lower right corners of the photograph) show little or no evidence of atrophy.

FIG. 3. No. 13696. Scharlach R stain. × 90. The epithelium of many tubules is laden with lipoid deposits which are black in the photograph.

FIG. 4. No. 13696. Phosphotungstic acid haematoxylin stain for fibrin. × 1170. The photograph shows a coagulated mass of exudate within the Bowman's capsule of one glomerulus. The exudate appears as a black crescent in the photograph, and is highly magnified.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

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THE EFFECT OF PARATHYROID EXTRACT UPON THE SERUM CALCIUM OF NEPHRECTOMIZED DOGS[1]

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Submitted for publication June 21, 1935

In order to explain the mechanism by which the well-known elevation of serum calcium following the administration of parathyroid extract is effected, several hypotheses have been advanced. Collip and his associates (1) (2) (3) have suggested a direct action of the hormone upon the osteoclasts of the bones. In supporting their view-point, they have recently described bone-changes in nephrectomized rats following the injection of parathyroid extract (3). Another group assumes the presence in the blood of a hypothetical substance which forms a calcium complex and thus increases the carrying power of the blood for calcium. Others have followed still a third line of thought. Albright (4) and Ellsworth (5) (6) (7) have emphasized in the human subject the regularity and immediacy of the outpouring of phosphorus in the urine following the administration of parathyroid extract. Recently in England, Goadby and Stacey (10) have also observed this effect of parathyroid extract. In their experiments it occurred regularly and, as in the studies reported by Ellsworth and Howard (7), even when there was no conspicuous change in the serum calcium level. Moreover, Goadby and Stacey found that lowering of the serum inorganic phosphate by the injection of glucose did not interfere with the outpouring of phosphorus in the urine produced by parathyroid extract. This seems to be a fundamental effect of the parathyroid hormone (6) (7).

The order in which the following observed changes appeared in the human subject (1) outpouring of phosphorus in the urine, (2) fall of serum inorganic phosphorus, (3) rise of serum calcium, (4) increase of

[1] This work was aided by a grant from the Council on Pharmacy and Chemistry of the American Medical Association.

calcium excretion in the urine, together with certain theoretical considerations, led to the development of the hypothesis by Albright and Ellsworth that the calcium changes following administration of the parathyroid hormone might be secondary to those of phosphorus. In testing this hypothesis the question at once arises—Will the parathyroid hormone produce an elevation of serum calcium when the loss of phosphorus through the kidneys has not previously taken place?

Two series of experiments are suggested to illuminate this question: first, observation of the effect of parathyroid extract in the nephrectomized dog, and second, the same test in the human subject with advanced renal insufficiency. The observations made upon the nephrectomized dog are the subject of the present communication; the second series, upon human subjects, will be reported in another paper.

Experimental

Three large dogs (M, P, D) were subjected to the following procedure. Each was given a small dose (2 cc.) of parathyroid extract (Lilly) as a test dose to ascertain whether the serum calcium of the dog responded in a normal manner to the hormone. The results are shown in table I. After an interval of five to seven days each animal was given nembutol (Abbott) prior to operation. Dog M received the nembutol subcutaneously. Satisfactory anaesthesia was not obtained and it was necessary to use ether in addition. Dogs P and D were given the nembutol intravenously, 0.43 cc. per kilo of the Abbott solution. The anaesthesia was excellent. (The same anaesthetic was used in the control dogs C, F, and K with very satisfactory results.) As soon as anaesthesia was induced both kidneys were removed.[2]

Immediately after the operation was completed 30 cc. of blood were withdrawn, part allowed to clot, and part preserved with heparin. The animals M, P, and D were given 7 cc. of parathyroid extract subcutaneously, 3.5 cc. in each groin. The animals were allowed

[2]We wish to thank Dr. Warfield M. Firor, Dr. Austin Lamont and their associates who performed the operations for us.

water *ad libitum*, but were not fed. Subsequent samples of blood (30 cc.) were taken at the times shown in table 2. Each sample of blood was analyzed by standard methods[3] for the following—hematocrit, NPN of whole blood, serum calcium, plasma inorganic phosphate, and plasma proteins. The results are shown in table 2.

As controls, three dogs C, F, and K, were anaesthetized and nephrectomized. Blood samples were taken in the same amount and at approximately the same times as in the other dogs, and were analyzed for the same constituents (table 3).

In the three dogs given parathyroid extract after nephrectomy a very definite rise in serum calcium occurred. This varied from 2.6 mgm. per 100 cc. in dog M to 4.0 and 4.2 mgm. per 100 cc. in dogs P and D. A moderately large dose of parathyroid extract (Lilly)

TABLE I

Preliminary test with 2 cc. parathyroid extract

TIME	SERUM Ca, MGM. PER 100 CC.		
	M	P	D
hours			
0	11.2	10.7	10.5
6– 7	12.4	12.7	
8			11.3
12–13	12.0	11.2	

was given, because it was considered that slight changes in serum calcium would not be significant, since some small alteration in calcium might occur as a result of the operation plus the removal of the fair amounts of blood necessary for the required analyses. That this precaution was justified is shown by the slight rise in serum calcium in the three control dogs, 0.9, 1.6 and 0.7 mgm. per 100 cc. blood respectively. The amount of calcium excreted in the urine per hour in the dog is so small that retention of that amount could not be held accountable by itself for the observed rise of plasma calcium. This rise in serum calcium in the control dogs may be explained by the operation of several factors. First, a certain degree of acidosis prob-

[3]Hematocrit by the Wintrobe, NPN by the micro-Kjeldahl, serum calcium by the Kramer-Tisdall, inorganic phosphate by the Fiske and Subbarow, plasma proteins by the macro-Kjeldahl methods.

TABLE 2

Blood constituents in nephrectomized animals after 7 cc. parathyroid extract

TIME*	SERUM Ca, MGM. PER 100 ML.			PLASMA INORG. P, MGM. PER 100 ML.			Ca x P PRODUCT			NPN			TOTAL PROTEIN, GM. PER 100 ML.			HEMATOCRIT					
																Plasma vol.			Cell vol.		
	M	P	D	M	P	D	M	P	D	M	P	D	M	P	D	M	P	D	M	P	D
hours																					
−1	10.3			2.3			23.7														
0	10.4	10.5	10.2		7.1	3.3		74.5	33.6	26	32	34		5.4	5.6	37	48	44	63	52	56
5		12.6			8.2			103			39			5.2			50			50	
6	12.9		13.4	4.1		7.1	53		95	33		47			5.2			47			53
10½		14.3			10.1			143			50			5.4			51.5			48.5	
10½			14.2			9.7			138			58			5.5			48			52
10½	12.9			4.8			62									33.5			66.5		
15	12.9			5.4						42											
24	10.6	14.5	14.4	8.2	8.4	13.1	87	122	188	78	84	108		5.0	5.3		56	52		44	48

* Time taken in hours after removal of both kidneys.

TABLE 3

Blood constituents in control animals, nephrectomized but not given parathyroid extract

TIME*	SERUM Ca, MGM. PER 100 ML.			PLASMA INORG. P, MGM. PER 100 ML.			NPN			TOTAL PROTEIN, GM. PER 100 ML.			HEMATOCRIT					
													Plasma vol.			Cell vol.		
	C	K	F	C	K	F	C	K	F	C	K	F	C	K	F	C	K	F
hours																		
0	10.0	11.3	10.5	6.8	4.5	6.1	29	40	45	5.8	6.3	5.7	50	48	45	50	52	55
5	11.1	11.5	10.5	8.8	7.1	12.6	41	60	63	5.7	6.0	5.3	49	53	41	51	47	59
10	10.9	12.0	11.4	10.1	7.5	10.0	64	69	90	5.7	5.8	5.3	51.5	50.5	48	48.5	49.5	52
24	11.6	11.7	11.4	11.5	8.2	11.8	100	112	124	6.3	6.1	5.9	54.0	53	49	46	47	51

* Time taken in hours after removal of both kidneys.

ably develops after nephrectomy, and this would tend to mobilize calcium into the blood. Second, and perhaps more important, is the removal of the fairly large quantities of blood necessary for the analyses. It has long been known, but without adequate explanation, that hemorrhage will temporarily relieve parathyreoprivic tetany. Possibly the removal of blood in some way mobilizes calcium. However, the difference in magnitude of the calcium rise in the dogs receiving parathyroid extract and that in the controls clearly demonstrates an effect of the parathyroid hormone in raising the serum calcium of the nephrectomized dogs.

In the control dogs C, F, and K the concentration of plasma proteins (table 3) fell somewhat at 5 hours and at 10½ hours and then rose to the starting value or above at 24 hours. When considered with the hematocrit readings there occurred from 0 to 10½ hours a slight increase in plasma volume with a slight dilution at the same time.

In dogs P and D the concentration of total protein in the plasma decreased at 5–6 hours, increased at slightly over 10 hours to the control level, and then decreased again somewhat at 24 hours (table 2). In both instances the plasma volume relative to that of the cells as shown by the hematocrit, increased slightly but steadily (table 2). The plasma protein values and hematocrit readings show that the steady rise in serum calcium concentration observed in dogs P and D was not an artefact due to hemoconcentration, but was indicative of actual mobilization of calcium into the blood.

The calcium rise took place in spite of a constantly rising inorganic phosphorus of the plasma. The rise in inorganic phosphorus was roughly parallel to that of the NPN.

An examination of the Ca x P product in table 2 shows that this increased to very high levels. Regardless of the value or significance of this rough product, it is nevertheless true that with Ca x P products in the region of 100 or more calcification in viscera such as kidneys and stomach, commonly called "metastatic" calcification, is very prone to occur. This serves to emphasize that the ability of the parathyroid hormone to bring about the removal of large quantities of phosphorus from the blood by the way of the kidneys and thus to maintain a low level of phosphate in the plasma, is a very important function. It prevents the inadvertent calcification of tissues which

would follow the mobilization of calcium and phosphate into the blood after parathyroid action.[4]

One question which might be anticipated is the following. In the absence of the kidneys, is there a marked outpouring of phosphorus into the gut under the influence of parathyroid extract, similar to the outpouring of phosphorus observed in the urine of the intact animal, and could such a loss of phosphorus be the cause of the mobilization of calcium phosphate from the bones? This problem is technically a very difficult one to approach directly. However, two points bear upon the question very definitely. First, although in the rat Pugsley (11) has shown that parathormone affects the fecal calcium excretion, Albright, Bauer, Ropes, and Aub (4) found that in the human being there was very little effect exerted by the parathyroid hormone upon the fecal excretion of calcium. With regard to phosphorus excretion, the latter workers (Albright *et al.*) showed that in comparison with the marked effect upon the urinary excretion of phosphorus, the parathyroid hormone had little effect upon the fecal excretion of this element. If the hormone were to produce a marked outpouring of phosphorus into the gut after nephrectomy, one would have to assume, provided that the observations made by Albright and others upon human subjects are applicable to the dog, that the removal of the kidneys had entirely altered the response of the gut to the action of the parathyroid hormone. This is, of course, possible. In the second place, it is obvious from an examination of the plasma inorganic phosphate figures in the dogs receiving parathyroid extract after nephrectomy that, if any increase of excretion of phosphorus into the gut took place, in any case it was not sufficient to prevent the very marked rise in plasma inorganic phosphorus which occurred in all three dogs and which was at least as marked as in the control

[4] Many writers have been misled by the results of repeated doses of parathormone described by Collip (8), by which the animal is poisoned. In such cases, *after* the calcium has reached a high level in the blood, the kidneys cease to excrete urine, the NPN and plasma inorganic phosphate begin to rise, and with a high blood calcium and inorganic phosphorus in the blood metastatic calcification occurs. It is important to distinguish between this effect of parathyroid overdosage and the effect of single doses of parathyroid extract. For full discussion of this point see Albright *et al.* (9).

animals. Since the blood inorganic phosphorus was not prevented from rising, it does not seem possible to attribute the mobilization of calcium into the blood after parathyroid extract in the nephrectomized animal to an increased excretion of phosphorus into the gut similar to the well-known increase in urinary excretion of phosphorus observed in the intact human being.

From the present experiments it is possible to conclude that in the dog the parathyroid hormone is able to mobilize calcium into the blood in the absence of the kidneys. The outpouring of phosphorus in the urine, commonly observed after the administration of parathyroid extract, does not seem, at least in the dog, to be necessary to the mechanism by which parathyroid extract raises the blood calcium. The workings of this mechansm, however, still remain in doubt.

SUMMARY

1. In three nephrectomized dogs the administration of parathyroid extract resulted in a marked rise in serum calcium.

2. A slight rise was also observed in nephrectomized animals which did not receive parathyroid extract.

3. Following nephrectomy, in both groups of animals the plasma volume relative to that of the cells tended to increase.

4. Observations of the plasma protein concentration as well as the hematocrit readings showed no hemoconcentration. The observed calcium rise may be interpreted as actual mobilization of calcium into the blood.

5. The inorganic phosphate of the plasma, as well as the NPN, as would be expected, rose. A very high Ca x P product resulted. The significance of this is briefly discussed.

It is concluded that the elimination of inorganic phosphorus in increased amount by the kidneys, commonly observed after the injection of parathyroid extract, is not necessary in the dog for the production by that extract of an elevation of serum calcium, since the elevation occurred in the absence of the kidneys.

BIBLIOGRAPHY

1. Thomson, D. L., and Collip, J. B.: *Internat. Clinics, Vol. IV, series 43, 1933.* The Hormone of the Parathyroid Glands.

2. Selye, H.: *Endocrinology,* 16: 547: 1932. On the Stimulation of New Bone-formation with Parathyroid Extract and Irradiated Ergosterol.

3. Collip, J. B., Pugsley, L. I., Selye, H., and Thomson, D. L.: *J. Exp. Path.*, **25:** 335: 1934. Observations Concerning the Mechanism of Parathyroid Hormone Action.
4. Albright, F., Bauer, W., Ropes, M., and Aub, J. C.: *J. Clin. Investig.*, **7:** 139: 1929. Studies of Calcium and Phosphorus Metabolism. IV. The Effect of the Parathyroid Hormone.
5. Albright, F., and Ellsworth, R.: *Ibid.*, **7:** 183: 1929. Studies on the Physiology of the Parathyroid Hormone. I. Calcium and Phosphorus Studies on a Case of Idiopathic Hypoparathyroidism.
6. Ellsworth, R.: *Ibid.*, **11:** 1011: 1932. Studies on the Physiology of the Parathyroid Hormone. V. Action of Parathyroid Extract on the Renal Threshold for Phosphorus.
7. Ellsworth, R., and Howard, J. E.: *Bull. Johns Hopkins Hosp.*, **55:** 296: 1934. Studies on Physiology of the Parathyroid Glands. VII. Some Responses of Normal Human Kidneys and Blood to Intravenous Parathyroid Extract.
8. Collip, J. B.: *J. Biol. Chem.*, **63:** 395, 439: 1925. The Extraction of a Parathyroid Hormone which will prevent or Control Parathyroid Tetany and which Regulates the Level of the Blood Calcium.
9. Albright, F., Baird, P. C., Cope, O., and Bloomberg, E.: *Am. J. Med. Sc.*, **187:** 49: 1934. Studies on the Physiology of the Parathyroid Glands. IV. Renal Complications of Hyperparathyroidism.
10. Goadby, H. K., and Stacey, R. S.: *Biochem. J.*, **28:** 2092: 1934. On the action of Parathormone.
11. Pugsley, L. I., *J. Physiol.*, **76:** 315, 1932. Effect of parathyroid hormone and of irradiated ergosterol on calcium and phosphorus metabolism in rat.

ADENOMA OF ADRENAL CORTEX SIMULATING PITUITARY BASOPHILISM (CUSHING'S SYNDROME)

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Submitted for publication May 24, 1935

Cushing, in 1932 (1), described twelve cases of "a peculiar and clinically unmistakable polyglandular syndrome," characterized "by a rapidly acquired plethoric adiposity affecting the face, neck and trunk, the extremities being spared. It is associated in women with hypertrichosis and amenorrhea. Other characteristic features are vascular hypertension, purplish striae distensae of the abdomen, and acrocyanosis with cutis marmorata of the extremities. It is often accompanied by hyperglycemia, occasionally by polycythemia, and a peculiar softening of the bones of the skeleton has been commonly found at autopsy. In its extreme forms, the malady has more often been encountered in young adults and the average duration of life in the fatal cases has been something over five years."

Cushing ascribed this malady to basophilic adenomata of the pituitary, but admitted that disorders "of a somewhat similar aspect may occur in association with pineal, with gonadal, or with adrenal tumors." Ten verified cases reported subsequently in the literature (2–11) prove that this syndrome may be produced by pituitary basophilism. But other cases (12, 4, 13), clinically indistinguishable, have been proved by autopsy to be due to adrenal tumors; and in still other instances (4) complete amelioration of the symptoms has been effected by operative removal of adrenal neoplasms.

It is obvious, therefore, that the clinical differentiation of the two conditions is difficult, as is illustrated further by the following case report.

CASE REPORT

Clinical history. R. P. M., 34, white housewife (formerly a nurse), admitted August 29, 1932, discharged January 26, 1933; died November 13, 1933. History No. 17,754.

Family and past histories non-contributory. Menses started at 13; periods were always irregular (6 to 8 week intervals), and flow scant.

Present illness: Four years before admission, the patient was well and healthy (Fig. I). She was said to have been tall, thin, and fair, and was able to work hard and efficiently. Her present difficulties started insidiously three or four years before admission, with a pustular eruption, unaffected by x-ray and vaccine therapy, which progressed to cover the face, neck, thorax, back and arms.

During 1930 the patient had no menstrual bleeding, although at the accustomed time for her periods her breasts would become painful and swollen for about a week. Her last period occurred two months before admission.

FIG. I. PHOTOGRAPH TAKEN TWO YEARS BEFORE ONSET OF DISEASE

In January, 1931, the patient suffered from a severe infection beneath the fascia lata of the right thigh. She was confined to bed with swelling of the thigh and knee, with a high fever. The lesion was incised and about a liter of pus withdrawn. Following this infection she underwent a gradual but steady change in appearance and disposition. Marked changes were noted in the integumentary system: The skin became coarse and thick; her once fair complexion changed to a red, plethoric hue, and a heavy growth of hair appeared on the face, axillae, in the pubic region and over the body in general. Her eyes became swollen, puffy and tender. Her face became round, and her neck and trunk larger. There was a definite and striking decrease in stature.

Coincident with these changes, she noticed a great increase in nervousness and irritability; she cried easily, was upset by trifles, and became impatient and fractious. In addition, because of weakness, it became more and more difficult for her to work, until at the time of admission she was quite unable to perform any physical labor.

Her physician reported that within a period of five or six weeks, her blood pressure increased from a systolic level of 100 mm. mercury to readings of 160/100, where it had remained for the two years preceding admission.

Physical examination: Temperature 37.2°C. Respirations 14. Pulse 106. Blood pressure 190/130. She was a very nervous white woman of about 33. The *face* (Figs. II and III) was markedly plethoric and florid, with prominent vein-markings on a background of dusky red skin. There was a profuse growth of beard, the hairs being deep black and of a maximum of 7 or 8 mm. in length. The eyelids were rather puffy, and the whole face seemed swollen.

She gave the definite impression of obesity. The fat was confined mainly to the face and trunk, the lower extremities being only slightly increased in size, and the upper extremities of about normal size.

The *skin* was rather oily. Scattered over the trunk, both front and back, were numerous papulo-pustular lesions which looked like those of an advanced acne. There was a mottled discoloration of both extremities. Over the outer sides of the thighs there were a few striae. The *mucous membranes* were normal in color, without jaundice or cyanosis.

The *hair* of the head was thick and black. There was a profuse beard. Axillary and pubic hair, although shaved, was evidently very thick. There was a heavy growth of lanugo all over the body, especially over the spine, lower lumbar region, and lower part of the abdomen. The distribution of pubic hair was typically masculine. The *nails* were rather thin and very rough and dry. (She said that almost every nail had fallen off at one time or another during her illness.)

There was slight bilateral pseudo-ptosis of the *eyelids* due apparently to puffiness. Slight photophobia was complained of. Ophthalmoscopic examination showed no evidence of increased intracranial pressure or optic atrophy. Visual fields normal on perimetric study. *Teeth* in rather poor condition, showing several extractions and considerable dental work. The *neck* was of the thick, short type, and was rather obese. The *thyroid* was not conspicuously enlarged.

The *chest* was thick; there was marked thoracic kyphosis, and the spine was slightly tender on heavy percussion. The *lungs* gave a normal percussion note, tactile and vocal fremitus, breath sounds, and descent of the bases. There were a few inconstant râles at both bases. The *heart* was at the upper limits of normal in size; sounds clear and forceful. Peripheral vessels not thickened. Blood pressure 190 /100.

Examination of the *abdomen* was unsatisfactory because of the very thick panniculus. *Pelvic examination* revealed an hypertrophied clitoris, a long and pointed cervix, and a uterus about twice normal in size.

FIG. II. PHOTOGRAPH TAKEN AT TIME OF ADMISSION TO THE HOSPITAL

FIG. III. PHOTOGRAPH TAKEN AT TIME OF ADMISSION TO THE HOSPITAL

The deep and superficial *reflexes* were normal.

Accessory examinations: Blood: R. B. C. 5,080,000; Hg. 16.1 gm. per cent (Sahli); mean hemoglobin content 3.02×10^{-8} mg.; W. B. C. 14,000 of which 86 per cent were polymorphonuclears. *Urine:* Occasional sugar 1 or 2 plus; occasional trace of albumin; no red blood cells at any time, either microscopically or chemically. *Wassermann* negative. *Blood chemistry:* Fasting sugar 80, 85, 114 mg. per cent; cholesterol 240 mg. per cent; non-protein nitrogen 36 mg. per cent; calcium 10 mg. per cent; phosphorus 2.8 mg. per cent; refractive index 1.3490.

Sugar tolerance test: Fasting, 100 mg. per cent; 1/2 hour, 185; 1 hour, 250; 2 hours, 275; 3 hours 140.

Studies of *calcium metabolism* over a three-day period showed: Intake (normal diet): Calcium 1.836 gm.; phosphorus 3.819 gm.; total nitrogen 50.76 gm. Urine: Calcium 0.631 gm.; phosphorus 3.175 gm.; total nitrogen 37.06 gm. Feces: Calcium 0.180 gm.; phosphorus 0.280 gm. Total excretion: Calcium 0.811 gm.; phosphorus 3.455 gm. Retention: Calcium 1.025 gm.; phosphorus 0.364 gm.

The basal metabolic rate was minus 8 per cent.

X-rays of the dorsal and lumbar spine showed marked narrowing of several of the vertebrae, with almost complete collapse of the eleventh dorsal vertebra. The long bones showed slight rarefaction. The sella turcica was indistinct, and the posterior clinoids were eroded.

The *Aschheim-Zondek* test for pregnancy, with morning specimens of urine, on immature mice was negative.

Course in the hospital: The patient was in the hospital for almost five months, during which time there was little or no change in her condition, except for increasing weakness. She was emotionally unstable and, realizing the hopelessness of her condition, was usually despondent.

All therapeutic efforts, including X-raying of the pituitary and parathyroids and injections of the growth-promoting extract of the pituitary (Squibb), were fruitless. She was discharged five months after admission. Shortly thereafter, she entered another hospital, where she remained until her death, ten months later. Details of the terminal phases of her illness are not available.

Anatomical diagnosis

Infected adenoma of the left adrenal cortex; obesity; hypertrichosis and hypertrophy of the clitoris (virilism); follicular atresia of ovaries; osteoporosis; generalized arteriolosclerosis; cardiac hypertrophy and dilatation; passive congestion of lungs and liver; thrombosis of branch of left pulmonary artery; hemorrhagic infarct of left lung; fibrinous pericarditis; hypoplasia of bone marrow; acne vulgaris.

Gross examination

The autopsy was performed 24 hours after death, the body having been left in a cool room in the meantime.

The *external appearance* agrees with the clinical notes.

The *pleural* and *pericardial* cavities contain no excess of fluid. The *lungs* are definitely collapsed, containing practically no air. The left lung contains a

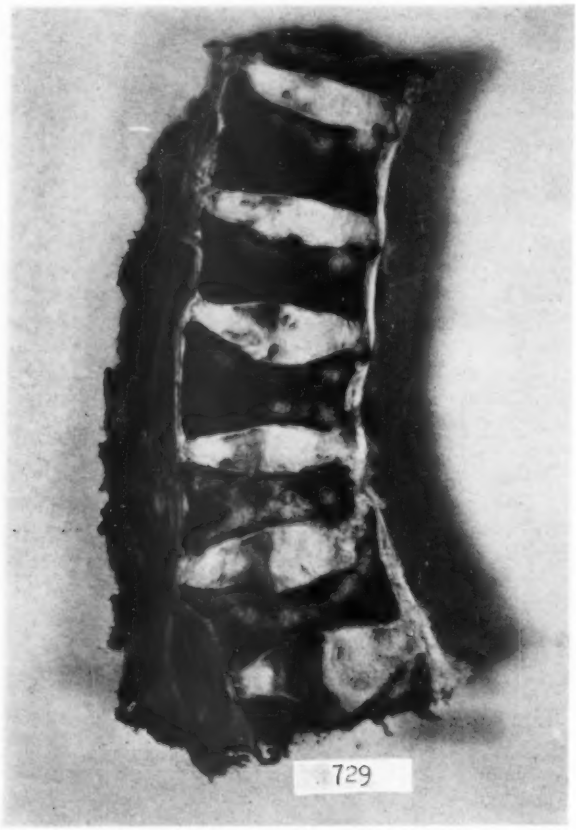

FIG. IV. GROSS SECTION OF SPINE, SHOWING SHORTENING OF VERTEBRAE AS RESULT OF OSTEOPOROSIS

Note hypoplasia of bone marrow

softened hemorrhagic infarct in the lower lobe with a thrombus in the corresponding branch of the pulmonary artery. The *heart* is enlarged, weighing 400 grams. All of the chambers are moderately dilated. A scanty fibrinous exudate is present

on the pericardium. The *gastro-intestinal tract*, *liver*, *spleen*, *kidneys*, *ureters*, *bladder*, *uterus*, and *brain* grossly are all normal.

Endocrine system: The *left adrenal* is replaced by a large, well defined tumor mass measuring 15 x 20 x 5 cm. The surrounding tissue and vessels are not invaded. On section the tumor is found to be made up of rather friable, mottled, yellow tissue. The lower half contains several large collections of pus. A small calcified area is present in the central portion. The *right adrenal* is distinctly atrophied, being reduced in thickness by almost one-half. The *hypophysis* is normal in size, shape and gross appearance. It measures 16 x 10 x 7 mm. The

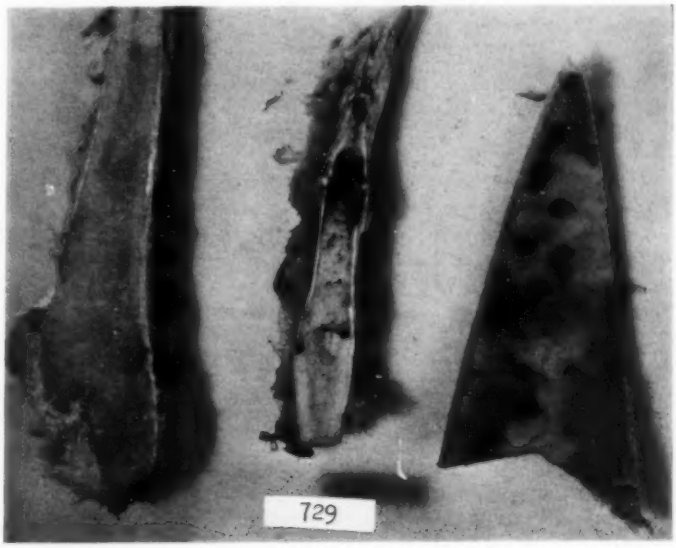

FIG. V. FIBULA, RIB, AND SKULL, SHOWING HYPOPLASIA OF RIB MARROW AND SOFTENING OF RIB AND SKULL

pineal body grossly is normal. On very careful search only one *parathyroid* body can be located. It measures 4 x 3 mm. The *thyroid* is normal in size and has the usual granular texture. No *thymus* tissue can be found. The *ovaries* are normal in size and contain a few follicles, smaller than usual; there are no corpora lutea. The *pancreas* is grossly normal.

Skeletal system: The *vertebrae* (Fig. IV) can be easily cut with a knife. Several in the thoracic and lumbar region are definitely shortened. The marrow, instead of being completely red, is largely yellow and fatty. The *ribs* (Fig. V) are also very soft with the marrow almost entirely fatty. There are numerous nodular thickenings, however, where the marrow is red. The *skull* is somewhat harder

than the rest of the skeleton, with numerous soft, translucent areas (Fig. V). The *posterior clinoid process* is very soft, being easily cut away with a knife. The *long bones* are fairly hard, with completely fatty marrow.

Microscopic examination

Staining methods: The hypophysis was divided and one portion fixed in formalin and another in Helly's fluid (Zenker-formol). The portion fixed in formalin was embedded in paraffin and serial sections were cut. These were stained lightly with alum hematoxylin followed by Mallory's connective-tissue stain (acid-fuchsin anilin blue) as suggested by Rasmussen (14). This method is simple and satisfactory, for it stains all of the cells of the anterior lobe differentially. The material fixed in Helly's fluid was cut serially and the sections were stained with acid fuchsin and methyl blue as suggested by Kindell (15) and with acid fuchsin and acid violet as suggested by Bailey (16), although Rigaud's fixation is preferable with the latter method. It should be noted that the so-called basophilic cells are not stained well by the common basic dyes but stain with certain acid dyes, such as acid violet and methyl blue, while the acidophilic cells stain with various other acid dyes and some neutral dyes, of which acid fuchsin is the best.

Fortunately for post-mortem material, a differential stain of the pancreatic islets was obtained with Bensley's neutral gentian and acid fuchsin.

The *tumor in the left adrenal* (Fig. VI) closely resembles the normal cortex. It is extremely cellular, containing many well formed capillaries but very little connective tissue. The cells are medium-sized and cuboidal or polyhedral in shape, some with deeply staining cytoplasm, some with pale and foamy cytoplasm. Nothing resembling chromaffin granules is present. The nuclei contain a moderate amount of chromatin and occasionally a nucleolus. No mitotic figures are seen. Occasionally two nuclei are present in one cell. There is widespread necrosis, with calcification in some areas. The necrotic areas are infected, containing a few leukocytes and many bacteria. Some, but not many, of the tumor cells contain lipoid.

The *right adrenal* has a thin cortex, composed chiefly of vacuolated, lipoid-containing cells. The medulla likewise is thin, no chromaffin granules being seen. One portion of the medulla is thickened owing to the presence of masses of large, polyhedral, rather pale cells, apparently medullary and different from the cortical cells.

In serial sections of the entire *hypophysis* no adenoma is found anywhere. The chromophobe cells are most numerous. Alpha (acidophilic) cells are in normal proportion, and a few beta (basophilic) cells are present throughout, but there is certainly no increase of the latter. (The sections of the hypophysis were studied by Drs. Cushing and Eisenhardt, whose observations agreed with ours.

The *pancreas* contains many areas of fat scattered among the parenchymal cells. The acinar tissue contains no zymogen granules, and some of the cells are

vacuolated. The islet cells are somewhat irregular and shrunken. Alpha, beta and indifferent cells are found in approximately normal proportions.

The *pineal*, *thyroid*, and single *parathyroid* glands are normal. The section from the region of the *thymus* contains nothing but fat. Both *ovaries* contain primordial but no well developed Graafian follicles. A few partially developed follicles are undergoing atresia with the formation of thin corpora albicantia.

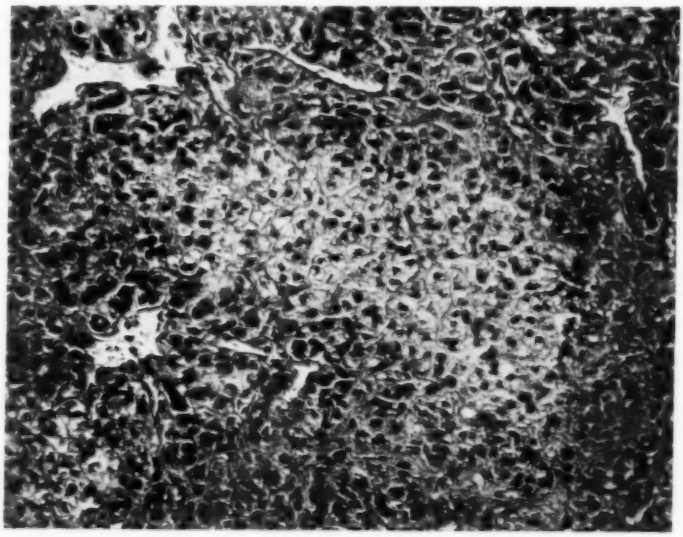

FIG. VI. MICROSCOPIC SECTION OF ADRENAL TUMOR, SHOWING BOTH PALE AND DEEPLY STAINING CELLS

Hematoxylin and eosin stain; low power

In the *ribs* and *vertebrae* there is marked, irregular atrophy of all bony trabeculae as well as of the cortex. In some areas there are very small cysts and thick areas of osteoid tissue. This change is present in all of the bones examined, including the head but not the shaft of the tibia. There is no great fibrous or granulation tissue accumulation such as is usually described in hyperparathyroidism (osteitis fibrosa cystica) or in osteomalacia, although there is a little fibrous tissue in some places. The *blood-forming tissue* contains both erythroblastic and leukoblastic tissue, the former predominating slightly. There is no extra-medullary myeloid tissue. An interesting observation in view of the high blood counts is a distinct hypoplasia of the bone marrow, there being many fatty areas in the ribs and vertebrae, while all of the tibia marrow is fat.

The *skin* contains many large infected comedones. These are so large as to be virtually small sebaceous cysts.

A hyaline and fatty thickening of the intima is seen in some of the *arterioles* of the kidney, pancreas, and ovaries. It is similar to that seen in cases of hypertension. The arterioles in the basal portion of the endometrium and adjacent uterine wall show reduplication of the elastic lamellae with reduction in size of the lumen. There are organized thrombi in several small arteries in the pancreas and a partially organized thrombus of the branch of the pulmonary artery leading to the left lower lobe. Corresponding to this there is an infected hemorrhagic infarct in the lung.

Both *lungs* are collapsed and contain many phagocytes with iron-containing pigment. No infection is present in other parts of the lung.

There is a fresh fibrinous exudate on the *pericardium*, which contains Gram-positive cocci. The *heart* contains a few areas of degenerating muscle, the largest of which involves one of the papillary muscles and is partially fibrotic. There are no changes in the cardiac vessels.

The *liver* shows changes characteristic of chronic passive congestion but of varying degree in various portions. The *spleen* seems normal.

DISCUSSION

Differential diagnosis: The problem of differentiation between pituitary basophilism and primary adreno-cortical lesions is difficult, if not impossible, in the present state of our knowledge. Osteoporosis, a feature to which we were at first inclined to ascribe differential value, is not pathognomonic, as is illustrated by this case and by observations of Robb-Smith (12) and Kepler (4).

Perhaps the most helpful differential feature is hypertrophy of the genitalia. According to Dr. Cushing (personal communication) this sign is frequently present in adrenal neoplasms, but has never been seen in the cases of pituitary basophilism so far reported. In Ashe's case (13), however, although an adrenal tumor was found at autopsy, the genitalia were normal. It is obvious, therefore, that the mere absence of genital hypertrophy is not diagnostic of pituitary basophilism to the exclusion of a primary adreno-cortical lesion.

Treatment: So long as the present difficulties in diagnosis obtain, treatment must necessarily be unsatisfactory. Kepler *et al.* (4) feel strongly that in the absence of a definite diagnosis these patients should be subjected to surgical exploration of the adrenals; and that even if a tumor is not found, the mass of hyperplastic cortical tissue should be reduced. The only objection to such a course is that,

contrary to the experience of these authors, most observers have found that patients showing this syndrome withstand operative manipulation very poorly. Even diagnostic pyelography has been known to be fatal, as in the case reported by MacCallum and others (17).

Hence, in view of the hazards incident to operative procedures, it seems safest first to try the effects of deep x-ray on the pituitary body. If this is not effective, then more radical diagnostic procedures are not only justified but indeed demanded—first pyelography and, as a last resort, surgical exploration of the adrenal capsules.

SUMMARY

A case is reported which was characterized by rapidly acquired, plethoric obesity, hypertrichosis, amenorrhea, hypertrophy of the clitoris, generalized osteoporosis, vascular hypertension, and moderate polycythemia.

This case was thought clinically to be an instance of pituitary basophilism (Cushing's syndrome), but at autopsy a large primary adrenal neoplasm was found, and the hypophysis was normal.

The questions of differential diagnosis and treatment are discussed.

BIBLIOGRAPHY

(1) CUSHING, HARVEY: Basophil Adenomas of the Pituitary Body and Their Clinical Manifestations. *Bull. Johns Hopkins Hosp.*, **50**, 137, 1932. Further notes on Pituitary Basophilism. *J. A. M. A.*, **99**, 281, July 23, 1932.

(2) MOEHLIG, K. C.: Basophilic Adenoma of Pituitary (Pituitary Basophilism). *J. A. M. A.*, **99**, 1498, October 29, 1932.

(3) BISHOP, P. M. F., AND CLOSE, H. G.: Case of Basophil Adenoma of Anterior Lobe of Pituitary: "Cushing's Syndrome." *Guy's Hosp. Rep.*, **82**, 143, 1932.

(4) KEPLER, E. J., AND OTHERS: Suprarenocortical Syndrome and Pituitary Basophilism: Presentation of Three New Cases. *Proc. Staff Meet. Mayo Clinic*, **9**, 169, March 21, 1934.

(5) BAUER, THEODOR, UND WASSING, HANS: Zur Frage der Adipositas Hypophysarea. (Basophiles Adenom der Hypophyse). *Wien. klin. Wchnschr.*, **26**, 1236–1243, 1913.

(6) BERGSTRAND, HILDING: Luteinisierung der Ovarien bei einem Falle von basophilem Hypophysenadenom mit Cushing's Symptomenkomplex. *Virch. Arch.*, **293**, 413–428, 1934.

(7) CRAIG, JOHN, AND CRAN, BRENNAN: Basophil adenoma of the pituitary gland. *Quart. J. Med., N. S.* **3,** 57–62, 1934.

(8) RUSSELL, DOROTHY S., EVANS, HORACE, AND CROOKE, A. C.: Two cases of basophil adenoma of pituitary gland. *Lancet,* **2,** 240–246, Aug. 4, 1934.

(9) MARBURG, O.: Über das basophile Adenom der Hypophyse, die cerebrale Fettsucht und die Pseudohypertrophie der Muskeln, *Arb. a. d. Neurol. Inst. a. d. Wien. Univ.,* **35,** 143–160, 1933.

RAAB, W.: Zur Symptomatologie des Morbus Cushing (Basophiles Vorderlappenadenom). *Wien. klin. Wchnschr.,* **47,** 1934.

(10) NOTHDURFT, KARL: Ein Fall von basophilem Adenom in der Neurohypophyse. *Frankfurt. Ztschr. f. Path.,* **10,** 91–100, 1912.

(11) RUTISHAUSER, ERWIN: Osteoporotische Fettsucht (Pituitary Basophilism), *Deutsches Arch. f. klin. Med.,* **175,** 640–680, 1933.

(12) LESCHER, F. G., AND ROBB-SMITH, A. H. T.: History of a Case of Carcinoma of the Adrenal Cortex with Cushing's Syndrome. *Proc. Roy. Soc. Med.,* **27,** 404, Feb. 1934.

(13) ASHE, J. R.: *Trans. Med. Soc. State of N. C.,* **81,** 258, 1934.

(14) RASMUSSEN, A. T.: The Percentage of the Different Types of Cells in the Male Adult Human Hypophysis. *Am. J. Path.,* **5,** 263, 1929; *ibid.,* **9,** 459, 1933.

(15) KINDELL, F. B.: Two Useful Staining Methods for Human Hypophysis. *Bull. Johns Hopkins Hosp.,* **53,** 56, July 1933.

(16) BAILEY, P.: Cytological Observations on the Pars Buccalis of the Hypophysis Cerebri of Man, Normal and Pathological. *J. Med. Res.,* **42,** 349, 1920–1921.

(17) MACCALLUM, FUTCHER, DUFF AND ELLSWORTH. *Bull. Johns Hopkins Hosp.,* **56,** No. 6 p. 350, 1935.